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"AN EMPIRICAL EVOLUTIONARY GENERALIZATION" VIEWED FROM THE STANDPOINT OF PHENOGENETICS

DR. RICHARD B. GOLDSCHMIDT
UNIVERSITY OF CALIFORNIA

IN his stimulating book on "Paleontology and Evolution," Simpson attempted to align the facts of paleontology with the Neo-Darwinian scheme of evolution. If I understand some of his general remarks correctly, he realized that many facts could as well be interpreted from the saltational point of view, although he has only harsh words for this type of evolutionary thinking. In reading this book critically I came to the conclusion that the opponents of Neo-Darwinism could easily use much if not most of the important material assembled to illustrate their heterodox point of view. In some chapters this might even be obvious to all readers. In the present paper, however, I do not intend to discuss this specific point; I wish, rather, to discuss only one single set of facts which, I think, may serve as an example to show how dangerous it is to look at evolutionary facts only from the standpoint of static genetics (*e.g.*, gene frequencies), forgetting that evolution is to a large extent also a problem of development.

On page 38 of Simpson's book the following statement is found:

> . . . exceptionally high values (of variability) usually indicate either characters for which the mechanics and function have been less rigidly integrated or characters that are degenerating and have lost all function. This last point, particularly important, has probably become apparent to every paleon-

tologist who has handled large collections. For instance, a functionless tooth, such as P_2 in Hoplophoneus, may vary from a well developed start to complete absence, not only within one race but also within one individual (*i.e.*, the left side may differ from the right side). In Philodus montanus, in which functional teeth have much the same variability as in other mammals (as exemplified above), the length of P_3, which is degenerating and losing function, has the high value $V = 18.5 \pm 2.8$ (V = Pearson's coefficient of variation). It is so commonly true that degenerating structures are highly variable that this may be advanced as *an empirical evolutionary generalization.*[1]

It may safely be assumed, as well from the general context in which this paragraph is found as from the entire tenor of the book, that the facts of retrogressive evolution contained in the quotation are accounted for by selection of small variants in an interbreeding population. It is my intention to show that certain facts of genetics and phenogenetics offer a very different and at least as convincing an interpretation.

In a number of former publications (*e.g.*, Goldschmidt, 1937, 1940; see also Villee, 1942), I pointed out the importance of the fact that some of the homoeotic mutants of Drosophila show an unusually high variability of the phenotype, including right-left asymmetry. This had been already noted by the discoverer of these mutants, Bridges, and Astauroff (1930) had made a special statistical study of this variability in the mutant tetraptera. The mutant tetraltera subsequently found by Goldschmidt showed the same phenomenon in still higher degree (details in Villee, 1942), and the last described member of this group, podoptera (Goldschmidt, 1945), has the highest degree of variability and right-left asymmetry. Viewing all the facts on record concerning the variability of these mutants (all of which transform a serial organ more or less into another member of the series, *viz.*, antennae into tarsi, halteres into wings, wings into halteres, labella into tarsi, wings into legs), we may stress the following points:

(1) Some mutants (or certain alleles of mutants having allelic series) show the character with 100 per cent.

[1] Italics mine.

expressivity, penetrance and bilateral symmetry, *e.g.*, one aristopedia allele found by the author has practically invariably always perfect antennal tarsi.

(2) Some alleles exhibit none of the homoeotic structure, being recognizable by other characters (*e.g.*, spineless allele of aristopedia with only bristle-effects, podoptera lines with only leg effects).

(3) Intermediate alleles exist in which there is a more or less extreme overlap with normal together with right-left asymmetry (*e.g.*, aristopedia, Bridges).

(4) Some mutants have been found only as lines with low penetrance, which include a high degree of right-left asymmetry, *e.g.*, tetraptera, tetraltera.

(5) In podoptera, penetrance is reduced to a low percentage of the individuals, the rest being normal. The variability of expression is extremely high, practically no two individuals being alike, and the right-left asymmetry is so extreme that almost all individuals have only one wing affected. If both are affected, the degree is asymmetrical.

(6) In all these cases a single recessive mutant (or one only slightly dominant) is involved in the action which produces the phenotypic effects, including variability. In podoptera, at least, the variability is unchanged in isogenic lines.

(7) This does not mean that genetic modification is nonexistent. Thus in tetraltera selection for higher penetrance is successful to a certain degree. Likewise, other mutant loci are known which enhance this penetrance (details in Villee, 1942). In podoptera, however, selection for plus modifiers has thus far been ineffective, and no clear enhancers have been located (unpublished work).

(8) Environmental modification of the percentage of penetrance is considerable in tetraltera and tetraptera, low temperature being especially favorable (see Villee, 1942). In podoptera there is only a small effect, if any, of temperature upon penetrance.

(9) Astauroff (1930) has shown by statistical analysis

of tetraptera that there is no right-left correlation, each wing reacting independently in the mutant. My own data on tetraltera and podoptera bear out the same conclusion.

Thus we face the fact that mutants exist which act in such a way upon the development of an organ that it is changed into another organ of a homodynamic series. Furthermore, in this case the effect may be complete or more or less incomplete, so as to result in a whole series of transitions from a normal organ to a completely different one. In addition, both sides of the body are affected independently. This means that all types of asymmetry may occur. We realize at once that, as far as phenotypes are concerned, the situation exactly parallels the example which Simpson used (see quotation above) to derive what he called an empirical evolutionary generalization. (The evolutionary difference between the two cases, *i.e.*, rudimentation versus unfolding of ancestral potencies, does not enter the discussion at this point.)

If we want to find out whether the two parallel phenotypic features may have an identical explanation, we must first consider a probable phenogenetic explanation of the genetically analyzed cases. We must consider first another group of facts which have been repeatedly discussed by me in this sense (*e.g.*, Goldschmidt, 1937). A number of mutants are known in Drosophila which have very little penetrance, showing their effect only in a few individuals of a homozygous strain not selected for plus modifiers. In such cases the lowest visible effect is usually unsymmetrical, *e.g.*, a nick in the wing, caused by the lowest members of the vg-series of alleles, and always occurring on only one wing, if the nick is very small. The same is true for certain silver alleles which produce a truncating effect in a pointed wing (see Goldschmidt, 1945a). The explanation proposed is that the decisive determining substance, whatever this means, is produced by the alleles in question in an amount near the threshold value, so that in the case of a low allele only a small section of the normal curve of variation of the determining stuff passes the

threshold value (*i.e.*, low penetrance); if there is no right-left correlation for the production of this substance, asymmetry occurs in the majority of cases. This general statement may be elaborated in detail in many different ways by assigning the variable to different developmental and determining processes (see below). Although the details might vary in different cases, I think that all

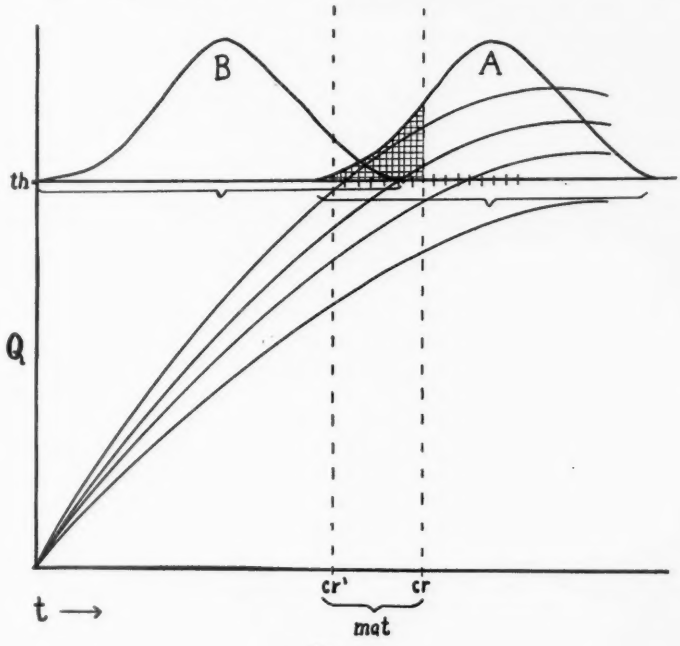

FIG. 1

workers in physiological genetics will agree that, in principle, the sort of happenings outlined will explain the facts.

Returning to the situation in the homeotic mutants, especially the ones with low penetrance, extreme variability, and asymmetry (*e.g.*, podoptera), we may describe the actual facts in terms of action of the mutant locus during development, using such necessary conceptions of phenogenetics as curve of reaction, quantity of active substances,

threshold of their activity, and time of action, in the diagram on page 309 (Fig. 1). In view of our ignorance of the actual processes, we add that the details may be conceived in different ways, but that some scheme of this type, involving the integration of different determining processes in development, must be at the basis of the situation. (Reaction, of course, always stands for unknown chains between the beginning and end; one possible variable, the time of onset of a chain, is left out for simplicity's sake.) The abscissa represents time of development (t), the ordinate the quantity (Q) of the substance which causes the action of the mutant upon the developing disc. The level th represents the threshold (concentration) at which the product of reaction can be effective. The line cr delineates the critical time in development after which determination can no longer be changed. The zone cr′–cr is a span in the development of the disc in which differentiation still proceeds before final determination. Let us call it a maturation zone (mat), in which some of the determining processes occur progressively so that the organ can be changed less and less by the mutant action with the advance of time. (The actual existence of such a condition has been demonstrated by Waddington (1940) for aristopedia; he explained it in terms of turning points; this explanation is implicitly contained in the present diagram.) Thus, if the threshold of action is reached before cr′ it means a perfect homoeotic mutant; action between cr′ and cr, from left to right, means increasing normality; and action to the right of cr a normal organ. The curves represent some of the possible chains of reaction producing the active end-product of the mutant, and the group of curves expresses the variability of these chains of reaction under the influence of the external or the genetic milieu. The curves of variation, A and B, show the frequency and range of this variation for two cases, assuming there is a normal curve of variation. In A only the shaded part of the curve is left of cr, and only a tiny section left of cr′. This is the situation in the

mutant podoptera: most individuals are normal (unshaded portion of A), relatively few (shaded area between cr′ and cr) show different degrees of effect, and very few (left of cr′) are podoptera. The curve B represents a case with no normals (the whole curve is left of cr) and only a few incomplete variants (between cr′ and cr). Any curve to the right of cr would represent 100 per cent. normals, in spite of the homozygous mutant, and any curve to the left of cr′ complete penetrance. Needless to say, genetic or environmental variation of the threshold, of the time of onset of the reactions, of the location of the critical period, etc., could accomplish similar results. Another feature, namely the variability of symmetrical podoptera phenotypes, can not be illustrated within the same two-dimensional diagram. In case of absence of correlation for right-left variation, the number of bilaterally affected individuals is the product of the two fractions:

$$\frac{\text{shaded area}}{\text{whole area A}}\ \text{left} \times \frac{\text{shaded area}}{\text{whole area A}}\ \text{right side of body.}$$

Complete symmetry is expected correspondingly to occur as the product of the right and left fractions of the curves left of cr′. Thus the actual facts on asymmetry are also contained in this diagram.

We now apply these facts and their phenogenetic interpretation to the paleontological example. The morphological and statistical resemblance of the two phenomena makes a parallel explanation reasonable. A mutant affecting a tooth (and we know of such simple mendelian mutants in man as well as in rodents, changing the type or causing the disappearance of teeth) might be of the type described or any of its possible variants, *i.e.*, acting upon a developmental system involving time of determination, thresholds, etc., of the general type as in Fig. 1. Thus the diagram might also explain the action of a mutant causing the rudimentation of a tooth. The line cr would indicate the time at which normal development of a tooth is determined if the genetically controlled reaction has reached

the proper threshold. Left of cr′ no tooth would appear (without deciding at this point whether this means lack of anlage, lack of adult tooth, etc.), and between cr′–cr different highly variable degrees of expression, *i.e.*, reduction, would occur. Variability and right-left correlation would completely parallel the Drosophila case. The same holds for genetic as well as environmental modification. The curves A, B, etc., again would represent different possible allelic conditions with their statistical consequences. One of the consequences might possibly be checked with the paleontological material, if it is sufficiently large: the larger the portion of the variants which fall within the zone cr′–cr the less probable are great right-left differences, and *vice versa*. (This has been found to be true for podoptera and tetraltera.) In concrete cases it might also be possible to check statistically the location of the curves A and B, or intermediate curves.

Assuming that our interpretation is correct, we might ask ourselves how regressive evolution takes place both genetically and phenogenetically. If the generalization contained in the title of this paper is correct, as Simpson states it to be, one single mutant with a type of action as discussed, causing any rudimentation, can produce all the conditions of variability, etc., described by Simpson. The complete disappearance of the organ might be produced in many ways, if we base our conclusions upon known genetical facts. Thus multiple alleles might be involved. Consider as an example the white series of Drosophila. Red may mutate to eosin, and this to white; or red to eosin, this to apricot, etc., or red in one step to white. Similarly in our diagram, normal might mutate to B via A, or directly to B, or by a series of steps. But this is not the only possibility. The same effect could be accomplished by mutations affecting completely different parts of the system, *e.g.*, speed of development, time of determination, etc. Such mutants may even have additional morphological effects, so that correlations appear between the disappearing part and unrelated structures. These proc-

esses may take place in one step, or in a series of steps, or even by a slow process of selection of modifiers. Which of these possibilities is realized must be decided, if this is possible, for each case individually. The genetic examples on which the present discussion is based suggest rather evolution by one or few steps; certainly there is no need for slow accumulation of small changes.

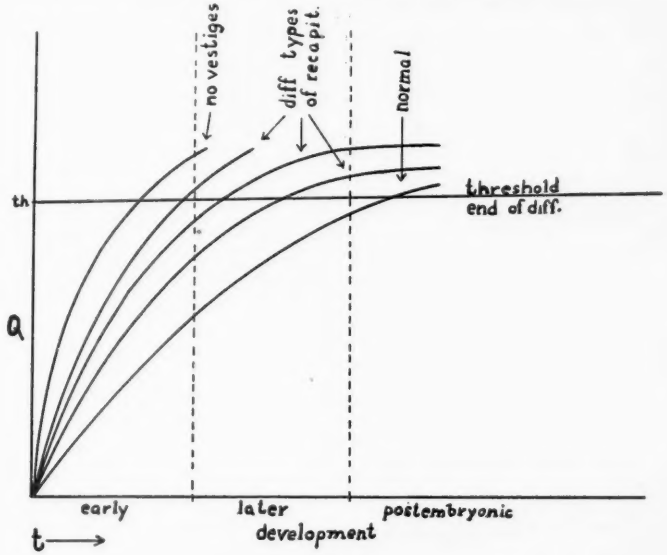

FIG. 2

There are a number of facts which are strongly in favor of the assumption that, in cases of retrogressive evolution, a phenogenetic system of the type discussed here is involved. A considerable number of cases are known which show that such evolution does not mean that genetic potencies disappear but rather that there is a change of timing within the developmental system, a change which might remain intact over eons. A genetic potency which has been relegated to stages of development is usually called a recapitulation, for example, the archaeopteryx stage in the development of the bird's tail, the embryonic

teeth of whalebone whales, segmented gonads in the development of some insects. These cases of regression with the original potency still visible in development may be described in terms of either change of relative velocity of differentiation below the threshold of normal growth or in terms of change of responsivity to the action of certain growth substances, or as any of many other available changes of a quantitative type affecting the part differentially from the whole without affecting the genetic potency

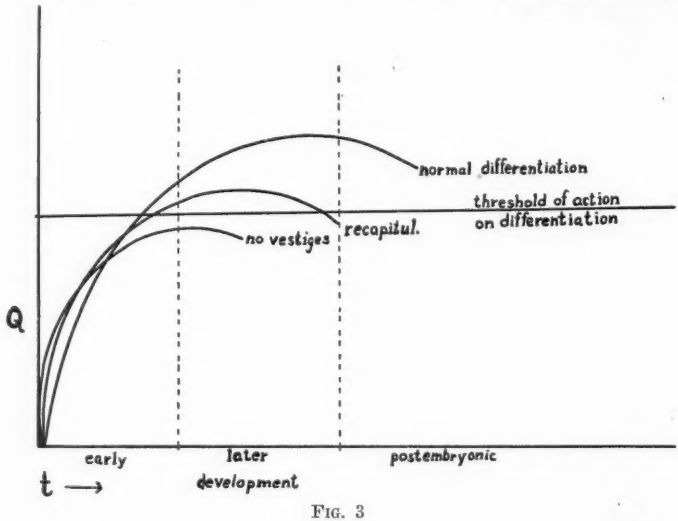

FIG. 3

to produce the part. From the standpoint of development, such changes may be described in a diagram (see Fig. 2) paralleling that of Fig. 1, which presents only one of many possible developmental situations of a related type. Only one other self-explanatory type of description is given in Fig. 3. Again we find in Fig. 2 time-of-development, curves representing the genetically controlled differentiation (including growth) of the organ, a threshold at which normal differentiation stops. The different curves represent normal development, normal development only during embryonic life, earlier embry-

onic recapitulation and complete absence of visible anlagen without absence of their potency. The actual presence of such a type of developmental system in the case of many evolutionary regressions is proven by the possibility of reawakening of the lost potencies (atavism), *e.g.*, the development of an external limb in a whale (Andrews) or upper canines in sheep (Benson). Reawakening would mean, in terms of physiological genetics, any environmental or genetic feature which shifts the curve of the determining process in question into harmony with those of the rest of the organism, in one of the many ways possible in the timed system. From the standpoint of genetics the phylogenetic retrogressive shift may be a single mutation, a series of multiple allelic mutations or a multiple series of small steps in the same direction, as already pointed out. Neither can be excluded *a priori*, but the facts concerning homoeotic mutants point strongly to single initial steps, as discussed elsewhere.

This is not the place to discuss in detail progressive evolution, with or without suppression of action of already present potencies, though the application of the same type of reasoning to this problem is rather obvious. But one example, to which Simpson devotes over two pages of his book, may be taken up because it presents a rather perfect parallel to the facts of retrogressive evolution just discussed. Simpson refers to such characters which, at a higher evolutionary level, become typical for certain genera but which have already appeared in the ancestors as occasional small "mutations." The example given is that of a small crest on the upper cheek teeth, which is typical for the genus Parahippus in the horse series and which subsequently, in the line to Equus, gradually becomes a large, essential element, the crochet. But already in Mesohippus, anteceding Parahippus, some individuals in the population contain a small crochet. This is interpreted as a mutant which has not yet become abundant or segregated. The descendants of these forms segregated into types with or without the character.

Other tooth characters also appear first as fluctuating varieties and later become universal. "Fluctuating" means that they vary in size and extent, *e.g.*, the number and location of affected teeth. Simpson concluded that the mutation introduced a new tendency in a complex developmental field, and that the phenotypic expression fluctuated under the influence of other genes related to the same field and of extrinsic factors influencing development. As I look at it the facts could be accounted for by postulating that, in genetic language, the evolutionary process begins with mutants of low penetrance and expressivity. It would seem that the simplicity of this explanation and its background of genetic facts makes it preferable to the complicated constructions of the Neo-Darwinian scheme.

Thus we meet here, in progressive evolution, the same type of mutant which was discussed and exemplified by the homoeotic mutants of Drosophila. The phenogenetic situation is the same as in Simpson's example, though evolution leads in the opposite direction. Must we now conclude that the evolution from the fluctuating crest (or incipient united metaloph, in another example) to the large crochet (or united metaloph) is the product of selection of many small additional mutants? The answer is the same as before: This can be the case; but it is also possible that the first mutant already had the potency for the maximum effect and therefore represented one large step which became, however, phenotypically visible only when another mutation in the developmental system changed the variables to a different timing.

One might object that the genetical examples on which we have based our discussion are rare occurrences, but I know of quite a number of comparable mutants, regarding some of which data can be found in a recent paper (Goldschmidt, 1945a). In two less popular species of Drosophila, actually the majority of mutants belong in this group (see Timofeeff-Ressovsky, 1931). But I do not intend to enter into more genetical details. The intention

of this discussion was only to show that a group of empirical facts of evolution, which look like an example for selection of small variations in a variable population, might be explained on a very different basis if facts of physiological genetics are taken into account.

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CHANCE AS A CAUSE OF CHANGES IN GENE FREQUENCY WITHIN PURE BREEDS OF LIVESTOCK[1]

PROFESSOR JAY L. LUSH

IOWA STATE COLLEGE

FOUR forces can change gene frequency. They are migration, mutation, selection and chance. A few introductory remarks about how the first three of these apply to the pure breeds of livestock may make clearer the part which chance plays.

MIGRATION

Most pure breeds of livestock in the United States have closed herdbooks. This means that no animal is admitted to registry unless both its parents are registered. Hence migration plays no part in these breeds, except as the occasional registration of a high grade by mistake or by deliberate fraud brings into the breed a sample gene from a population in which the gene frequencies were somewhat different.[2]

A few breeds, mostly new ones just getting well established, do admit high grades to registry. In some other countries, notably some of the importing ones, there is even less registration of high grades than in the United States. In still other countries the registration of high grades is permitted in most breeds. A common rule in such cases is that males are registered only when both parents are registered but that females which have three or four topcrosses of registered sires may be registered

[1] This paper was prepared for the program on Gene Frequencies planned for Cleveland in September, 1944, but later cancelled. Journal paper No. J–1299 from the Iowa Agricultural Experiment Station. Project 33.

[2] For some breeds importation from the native land into the United States still continues. In this sense (migration from one country to another instead of migration from outside the breed into it) migration can, of course, change the gene frequency in the breed in the importing country if the gene frequency there differs from that in the exporting country.

if they are approved on individual inspection or can pass certain production tests.

Since the immigrants which succeed in entering a breed must already be high grades, either in the breeds in which this is openly permitted or in the breeds in which it is not permitted but occasional admissions do happen by mistake or fraud, and since the proportion thus admitted is always small except sometimes when the breed has just recently been organized, little change in gene frequency in the pure breeds of livestock actually does result from migration, either from other breeds or from the unorganized bulk of the species.

Mutation

Because of its rarity, mutation can be almost entirely neglected as a force for changing the composition of a breed within a few generations. Forces which change a breed only infinitesimally within a few generations are of little interest in animal breeding, since such breeding is conducted to satisfy the wants and aspirations of man. Man is usually in a hurry and individually his time on earth seems short. Forces or processes which change things with almost imperceptible slowness may appeal to his imagination and curiosity as a philosopher, but they do not stimulate his efforts as a laborer or attract his resources as an investor.

Mutations do occur, of course, but their importance lies not in the extremely tiny changes in gene frequency which are thereby produced directly, but in the new opportunity the mutations then offer for chance or for selection to increase the frequency of a gene which was hitherto absent or extremely rare in the whole breed. The production of polled varieties of cattle, such as the Polled Herefords, within pure horned breeds is an example. The gene for polledness had first to be introduced by mutation (assuming that it was not introduced by undetected immigration) but the subsequent rapid increase in its fre-

quency was accomplished nearly altogether by selection. Recurrence of mutations can have played only the tiniest part in increasing the frequency of the new gene after it had already been introduced.

Selection

Selection is considered by most animal breeders as the main force at their command for changing the composition of their individual flocks and herds or of their whole breed. Some even imply that it is the only force, or at least the only one important enough to consider. A full statement concerning the principles and effectiveness of selection would be intricate and long enough to fill whole books. Only enough need be said here to distinguish clearly between selection and chance as sources of changes in gene frequency.

Selection is the existence of differential rates of reproduction, whereby the average number of offspring left per individual of one kind is larger than the average number left per individual of another kind. If, on the average, individuals which possess gene *A* leave more descendants each than those which do not, then the frequency of gene *A* will tend to increase as long as this situation prevails. It matters not whether the causes of these differences in rates of reproduction are man's preferences or natural conditions which permit individuals of one kind to survive and reproduce at higher rates than individuals of another kind.

Neither is it necessary that the difference in reproductive rate be true *for every individual* of each kind. There is plenty of room for chance and accident to cause some individuals of the generally more favored kind to leave no offspring and for some individuals of the less favored kind to produce many offspring. Such chances and accidents reduce the intensity of selection—and therefore are of much practical importance because they lower the rate of change—but they do not alter the direc-

tion of that change. All that is necessary for selection to change the frequency of gene *A* is that those which possess *A* and those which do not possess it shall have some difference in the *average* number of offspring which reach breeding age.

Selection can be practiced (*i.e.*) outwardly distinguishable kinds of individuals can differ in their average reproductive rates and yet not change gene frequency. This can happen either (1) when there really is no difference in the frequency of gene *A* in the favored and in the disfavored groups or (2) when such a difference exists but the resulting tendency of selection to change gene frequency is balanced by opposing forces, such as mutation or migration. Several genetic conditions or combinations of conditions can give rise to the first circumstance. Notable among them are: (1) the differences between the outwardly distinguishable types or kinds may be caused wholly by environmental differences; (2) that the most favored kind is a heterozygote; (3) that the favored kind is an intermediate; or (4) that the conditions under which the population lives are varied enough that there is room (an ecological niche) for at least a few of each kind to prosper, somewhat as in the economics of human societies there is room for a few but not too many shoemakers, a few lawyers, a few dentists, etc.

Thus there is abundant ground for denying that selection is universally effective at all times, even when freely granting that under most conditions selection may be the most potent single force for changing gene frequency. Unlike chance, which is as likely to raise as to lower the frequency of a gene, selection is a biased or steady force tending to carry gene frequency toward some value which remains fixed as long as conditions for that population do not change. Of course conditions never do remain absolutely unchanging over long periods of time. Therefore selection may rarely succeed in bringing a popula-

tion into complete equilibrium. Selection intensities and even directions are likely to change at least a little as environmental conditions or the breeders' goals or the frequencies of other genes change. Hence the equilibrium point toward which selection tends to carry a gene frequency often moves about with time and with ecological location.

How Chance Can Change Gene Frequency

Gene frequency can change just by chance in the Mendelian sampling which takes place when one generation replaces another. The N individuals in a generation just reaching breeding age were constituted by the coming together of $2N$ gametes from the parental generation. For each pair of genes the new generation is a sample of size $2N$ drawn from the parental generation. From the new generation itself another sample of gametes will be drawn to constitute the next generation. Thus the procession of the generations is a continuous chain of sampling from a sample which itself is a sample from a sample from a sample, etc.

Because of the chain-like or tandem nature of this sampling, the changes due to sampling have opportunity either to cancel each other or to accumulate. Naturally they will sometimes do the one and sometimes the other. The relation between the composition of successive generations is not quite the same as that between two independent samples from the same universe. Instead the composition of one sample is the composition of the universe from which the next sample is drawn.

If the sampling from the parental generation were truly random, the analogies with sampling from a thoroughly mixed population of balls in an urn might be entirely accurate as a model for measuring the amount of change in gene frequency which chance in this sampling could be expected to cause. In that case, because of the binomial nature of Mendelian inheritance, we could

write the variance of gene frequency in one generation from this cause as $\frac{q(1-q)}{2N}$ where q is the gene frequency among those reaching breeding age in the parental generation and N is the number of individuals which reach breeding age in the next generation. Over a period of t generations the variance due to this sampling would be t times this amount, except as the variance is damped down when q nears its limits of 1.0 or zero.

This simple situation may perhaps be approached rather closely in some populations. However, the sampling often is not random and no biological population is as structureless as a population of thoroughly mingled balls in an urn can be. Even in a population mating entirely at random, there are the family relationships which we describe verbally as sets of full sibs, half sibs, cousins, etc., with gene frequency varying at least a little from family to family. Especially in the pure breeds of livestock several conditions are likely to be important enough to make the simple formula for variance of a binomial distribution underestimate seriously the amount of change in gene frequency which will result from chance.

Variations in the Number of Offspring Per Parent

Concerning the number of offspring each potential parent will have, the simplest model is the case of entirely random sampling from the parental generation. This is to assume that each gamete is as likely to come from any one potential parent as from another. Then the potential parents would be distributed in a Poisson series with respect to the actual number of offspring each has. Thus, in a population static in numbers, the mean number of offspring reaching breeding age, per individual which reached that age in the parental generation, would be two[3]

[3] In cross-fertilizing organisms, since each offspring has two parents. In self-fertilizing organisms an average of one offspring per parent would be adequate, but each such offspring gets two gametes from its parent. In either case an average of two gametes per parent is required.

and the fractions of the parental generation which would achieve each of the possible discrete numbers of offspring would be as follows:

NUMBER OF OFFSPRING EACH	FRACTION OF THE PARENTAL GENERATION
0	.135
1	.271
2	.271
3	.180
4	.090
5	.036
6	.012
7	.003
8 and more	.001

Equal probability of becoming a parent is thus by no means the same thing as requiring that each individual actually have the same number of offspring. If equal probability of becoming a parent actually did prevail, the $\frac{q(1-q)}{2N}$ formula would describe the role which sampling variations play in changing gene frequency in one generataion. Wright (1940) has explored the effect of deviations from this Poisson distribution. He relates the actually observed or census number (N_0) in the population to the "effective number" (the number which would give the same amount of chance variation in gene frequency if sampling were entirely random) in the following formula: "Effective number" $= \frac{4N_0 - 2}{2 + \sigma_k^2}$ where k is the actual number of offspring per potential parent. If the distribution is Poisson, the variance of k is equal to 2 and the effective number and the actual census number are practically identical. If the variance is larger than in a Poisson distribution, the effective number will be less than the census number. It will be vastly less if the variance of k is extreme. If there were biological or physical circumstances compelling every potential parent to have exactly two offspring reach breeding age, the

variance of k would be zero and the effective number would be nearly double the census number.

For the variation to be less than Poisson is rare and requires that the probability of a potential parent having another offspring reach breeding age shall diminish sharply with each additional time it does achieve parenthood. The only actual circumstances which cause this condition to be even remotely approached are such minor ones as that among mammalian females the beginning of one pregnancy ordinarily precludes the beginning of another until the first has been terminated and in some species makes another pregnancy unlikely until the nursing period following the first one is completed, or that among birds like pigeons or doves a pair with two eggs already in the nest ordinarily have zero probability of producing any more eggs until these two have been hatched and the young reared.

Much more common are circumstances under which the probability of becoming a parent again increases with each time that is achieved. For example, some individuals among those reaching breeding age are genuinely sterile. The real probability of these having offspring is zero but, since they can not be identified in advance, they have to be included when computing for all those which reach breeding age the average probability of becoming a parent. They would be excluded when figuring the probability that an individual which already has one offspring will also have a second one.

Correlation between the Fates of Relatives

In nature as well as on farms many animals rear their young in broods which stay more or less together for part of their life cycle and are to some extent subject to the same accidents, infections and other vicissitudes. A covey of quail, a swarm of bees, a flock of sheep, a hen's brood of chicks, a litter of pigs or the swarm of minnows which hatch from a single batch of eggs are examples. If

a predator or infection or some accident destroys one, the others have more than an average probability of being destroyed also. If one of them survives to reach breeding age, the other members of the brood have a higher-than-average probability of surviving also.

This kind of circumstance increases greatly the variation in number of offspring per parent. In extreme cases, as with some fish or insects, the great majority of the potential parents may have no offspring reach breeding age while a very few may have 30, 50, 100 or even more. Such a condition gives k an extremely large variance and makes the "effective number" of individuals in the breeding population vastly smaller than the census number. It is the number of a parent's offspring which reach breeding age—not the number *born* to that parent—which determines how many of the $2N$ gametes which make up the next generation came from that parent.

In all pure breeding, aside from a few exceptional cases in which "flock registration" is practiced, the individual pedigrees are known and some attention is paid to the merit of relatives in deciding which animals are to be used at all in pure-bred herds and which of those used shall be continued in use long after their first offspring are available. Paying attention to the progeny test, as is widely recommended for deciding about the further use of a sire or dam or whether to use their earlier offspring extensively for breeding, is an example of how a considerable correlation between the fates of relatives is brought about.

Perhaps the effect of this attention to family and pedigree is all included in the formula already mentioned for relating the "effective number" of individuals in the breeding population to the actual number. If there is any inadequacy in that formula, it seems to devolve from the fact that attention to the family often lasts for more than the one generation described in the formula. Chance and continued selection prevent many actual parents from

becoming grandparents, many actual grandparents from becoming great grandparents, etc.

Emphasis on One Ancestor in Selections

Breeders usually try to get for their next herd sire a son, grandson or other close relative of the living or recently dead sire or dam which they consider best in the breed. If many of them thus admire the same ancestor, the genes of that animal become widely spread through the whole breed by the extensive use of many of its close relatives. The frequency of each gene in the whole breed is moved part way from wherever it was toward whichever one of the three possible values (zero, .5, or 1.0) it had in the famous animal.

Here it is easy to confuse the effects of selection and those of chance variations. To some extent an animal becomes famous in the first place because of the genes it had. To that extent the changes in gene frequency in the whole breed which are caused by the extensive use of the animal and its relatives are the result of selection. But even the most famous animal also has many genes which are nearly neutral and at least a few genes which are even harmful in their effects. The frequency of these genes also will be increased by the extensive use of that animal. The sons and other relatives of that animal will not all have the genes which helped make it famous. Using them extensively, merely because they are relatives, opens the door wider for large amounts of chance change. If the number of currently famous animals were always large, the average frequency of each neutral gene among them would be nearly identical with the average frequency of that gene in the whole breed and the average frequency of each harmful gene among them would, of course, be lower than in the whole breed. In such a case the changes in gene frequency produced by the extensive use of such animals and their relatives would be the steady changes expected to result from selection. But

when there are only a few of these famous animals the chance presence or absence in them of a gene which is rare or abundant in the breed is not fully cancelled by the chance absence or presence of the same gene in other widely popular animals. It is the uncancelled remainder of these chance differences between the gene frequency in the famous animals and the gene frequency in the whole breed which causes the extensive use of the close relatives of a few animals to produce the truly chance changes in gene frequency in the whole breed. Obviously the magnitude of such chance changes is enhanced more and more as the number of animals on which attention is focussed becomes smaller or—which is the same thing—the larger the fraction of the whole breed's inheritance which comes from one or a very few ancestors.

Functional Stratification of Pure-bred Herds

Most pure-bred herds fulfil the main economic function of the pure breeds—producing sires for use in the grade or commercial herds—while a few "breeder's herds" supply most of the males which are used in ordinary pure-bred herds. In order to sell many sires into other herds of pure-breds, it is usually necessary to maintain a considerable program of advertising, showing and sales promotion. To reduce the overhead cost per animal sold, those who try to sell sires at all into other pure-bred herds are under pressure to sell into such herds as many as possible of the males born in their herds.

The existence of this economic threshold which must be surmounted to become a breeding herd, in addition to the attention which most breeders pay to pedigree, brings about a high correlation between the fates of sibs. If a sire or dam happens to be located in a breeder's herd and is thought good enough to have a son or daughter used in one pure-bred herd, it is likely to have other sons or daughters put to such use also. This is accentuated by advertising and the arts of salesmanship. As far as con-

cerns merit (either outward or genuinely genetic), the distribution of herd averages from best to poorest is an approximately continuous one. Since it is primarily the economic circumstances of advertising costs and showing costs which interpose a partial threshold and tend to introduce a little discontinuity between breeder's herds and multiplier's herds, the stratification will have small (although not quite zero) correlation with the genetic merit of the animals. Therefore the reduction in the effective size of the population which such stratification causes will largely increase the chance changes in gene frequency instead of the systematic changes caused by selection, although there will be some of the latter to the extent that stratification is based on apparent merit of the animals in the herds.

The breeder's herds constitute a breed within a breed except that immigration into them from the rest of the breed can and does take place when their owners purchase unusually promising animals found in the multiplier's herds. Prevailingly the rest of the breed is being graded up toward the composition of the breeder's herds. The breeder's herds are analogous to the "centers of radiation" often mentioned in discussions of organic evolution.

One form of stratification is sometimes based directly on the supposed merit of the animals. Thus it is common for the owner of a large ranch to maintain a separate "stud flock" composed of his very best males and females. From these he produces the sires which he uses on the rest of his ranch to produce the lambs or calves he intends to sell on the commercial market. Often this stud flock is itself headed by sires he has purchased from some famous "breeder's herd."

Non-random Mating

Isolation (complete or partial) by distance or other physical barriers is not often important in farm animals.

Yet there is some of it. A breeder purchasing new stock naturally has some inclination to purchase from the herds which he knows best. These are likely to be near at hand. The consequences seem to be the same as Wright (1943) has discussed for species in nature; namely, some tendency to differentiate into local or regional subgroups varying somewhat from each other in gene frequency. This formation of local subgroups is partly cancelled by the equally widespread human tendency to regard with favor that which comes from a distance. One need only mention the glamour which attaches (or used to attach) to the word "imported" as a sales appeal for many commodities. This exists strongly in those introduced breeds for which importation has not come completely to an end. In such breeds selling the young males may be easier if the owner can advertise that they are by an "imported" sire. The balance between these opposing tendencies to differentiate the breed into local races by purchasing stock only from herds near at hand, and the opposite tendency to obliterate the incipient local differences by going far to purchase new stock sways back and forth from time to time and from breed to breed according to current advertising fashions, economic encouragement to experiment with distant stock, the historic background of the breed, etc. Stonaker (1943), studying this in the Aberdeen-Angus breed, estimated that only about one tenth of the increase per generation which he observed in the inbreeding coefficient could be attributed to isolation by distance.

Little really intense inbreeding is practiced in producing pure-bred livestock. Yet in each breed a few breeders are likely to be line-breeding to some animal famous in the breed a generation or two earlier. If several are doing this but are directing their efforts toward different ancestors, this tends to form distinct subgroups within the breed. Within each subgroup the gene frequency tends to drift up and down rather violently (on

account of the emphasis on one ancestor and the rather small numbers within each group) but the drift within one such group will be uncorrelated with that in other groups in the same breed. If the number of such groups is large, the effect on the average frequency of a gene in the whole breed will be small and is already included in the discussion of the results of most breeders striving to get sires which are sons or grandsons of a few currently famous sires. However, this forming of several rather unrelated line-bred families within the breed does set the stage smoothly for selection between families and makes it easier for such a family to change the gene frequency of the whole breed sharply if that family establishes a reputation as the best family in the breed and a pedigree "boom" ensues. In such a case there follows a period of grading the whole breed up toward the popular family. Examples of how this has at times worked rather powerfully are the trend to "straight Scotch" Shorthorns early in this century and toward "straightbred Anxiety 4th" Herefords after about 1912.

The Actual Amount of Random Change in Gene Frequency

The inbreeding coefficient is a measure of the amount of random change in gene frequency which is likely to have occurred in a pure breed of livestock. Wright (1931) has demonstrated that the primary effects of inbreeding on the genetic structure of an inbred line are identical with and flow from the random drift of gene frequency due to chance in the Mendelian sampling by which one generation replaces the preceding one. He and McPhee (1925) devised a method for sampling whole breeds from this point of view, whereby the inquiry into what has happened in a whole breed would not be blocked by the sheer physical impossibility of studying any considerable number of complete pedigrees. McPhee and Wright (1925) used this method to study the Shorthorn

breed in Britain. Workers in several other places have studied one or more breeds each. The results of all these studies known to the writer are summarized in Table 1.

The increase per generation in inbreeding seems re-

TABLE 1

INBREEDING AND SUBDIVISION OF THE PURE BREEDS OF LIVESTOCK

Breeds studied	Author[1]	Inbreeding found (percent)		"Effective number" of males in the population[3]	Index of subdivision[4]
		Total	Per generation[2] in recent years		
Cattle:					
Shorthorns in Britain	McPhee and Wright	26.0	0.6	21	1.0[5]
Jerseys in Britain	Smith	3.9	0.2	62	
Ayrshires in Britain	Fowler	5.3	0.5	25	
Holstein-Friesians in the United States	Lush et al.	4.0	0.4	31	2.3
Brown Swiss in the United States	Yoder and Lush	3.8	0.5	24	1.7
Herefords in the United States	Willham	8.1	0.6	20	1.8
Aberdeen-Angus in the United States	Stonaker	11.3	0.3	41	1.6
Brown Swiss in Switzerland	Sciuchetti	1.0	0.2	75	0.6
Telemark in Norway	Berge	7.0	0.5	25	
Swine:					
Poland-China in the United States	Lush and Anderson	9.8	0.6	20	1.3
Landrace in Denmark	Rottensten	6.9	0.5	27	0.8
Sheep:					
Rambouillet in the United States	Dickson and Lush	5.5	0.7	18	3.9
Hampshire in the United States	Carter	2.9	0.9	14	11.6[6]
Horses:					
Clydesdale in Scotland	Calder	6.2	1.2[7]	10[7]	
Thoroughbreds in the United States	Steele	8.3	0.6	20	1.0
Standardbred (select sample)	Steele	4.0	0.6	20	0.6
Saddle horse in the United States	Steele	3.2	1.1	11	1.0

[1] For references see Stonaker's report (1943) or Carter's (1940) or Willham's (1937) or page 288 in the second edition of Lush: "Animal Breeding Plans."

[2] This is approximate. Several of the studies did not state the average number of generations involved. In some the figure includes only the changes in recent years; in others it includes the foundation period when (generally) the inbreeding was more intense.

[3] These are the figures in the preceding column, converted into the N of the $1/8N$ formula which describes the random sampling variance of gene frequency which would occur in a population mating at random, with the probability of becoming a parent uniform among the males and also uniform among the females but there being far more females than males in the breeding population. N is the number of males which, under those conditions, would have caused the observed rate of increase per generation in inbreeding.

[4] This is $\frac{\text{inbreeding found}}{\text{inbreeding expected}}$ where the inbreeding expected is $\frac{R}{2-R}$ and R is the average relationship between random members of the breed.

[5] In recent years. It was 1.3 at the two earliest dates studied.

[6] But this is based on a very small *inter se* relationship which has a high sampling error.

[7] Assuming that the average generation interval is 11 years, which might be considerably in error here. Steele found it to be nearer 12 years in light horses.

markably uniform in view of the diversity of species and regions represented. The figures in the column headed "per generation in recent years" when multiplied by $\frac{q(1-q)}{.100}$ are estimates of the variance of gene frequency from chance in one generation of Mendelian sampling. If we put these in terms of standard deviation, they yield about .02 to .05 as the standard deviation of the chance changes in gene frequency from one generation to the next when q is near .5, but of course considerably less when it is near 1.0 or zero.

These are large changes, especially when it is remembered that they apply to all genes, regardless of whether the gene has a large effect or a small one, and will sometimes accumulate by going in the same direction for several successive generations. It may well be doubted whether selection is as strong in any one generation, except for genes which have pronouncedly desirable or undesirable effects.[4] However, selection is a steady pressure (unless the goal or the environment change) and becomes stronger the farther gene frequency wanders from its equilibrium value, whereas these chance changes go sometimes in one direction and sometimes in the other and therefore often cancel each other. Selection may rarely lose control completely of genes with major effects, even though this control is loose enough that the frequencies of these genes can at times wander widely from their equilibrium value.

The considerable differences in the figures for total inbreeding observed in various breeds arise partly from the pedigrees being traceable to more remote dates in

[4] The primary act of selection must be a choice between individuals rather than between genes. If the apparent merit of the individual can be affected by dozens or even hundreds of genes for each of which the breed is not entirely homozygous, the intensity of selection between individuals must be divided and diluted among these genes and, even when very intense between individuals, must become very weak selection as between the average gene and its allele.

some breeds than in others. That the figure for total inbreeding in the Shorthorns is so high may be due largely to the historical accident that many lines of ancestry in this breed are known back to more than 30 years before the first herdbook was assembled. Had it been impossible to trace Shorthorn pedigrees farther back than 1810 (twelve years before their first herdbook was printed), the total inbreeding found would have been only about 11 per cent., and if traceable only to 1825 would have been only 7 or 8 per cent.

In many breeds the auxiliary history (*i.e.*, facts concerning the location and ownership of the herds, traditions about the breeding practices used, the number of animals in founders' herds, etc.), indicates that there was more inbreeding during the formative period when the breed existed in only a few herds than later when it had become popular and had expanded greatly in numbers. In breeds in which the pedigrees can not be traced into this formative period, it is of course impossible to measure how much inbreeding happened then. That it always took some such inbreeding to weld a breed together in the first place and to set it apart from the rest of the species seems plausible on genetic grounds, but the actual historical evidence to prove this beyond doubt is lacking in many cases. In some cases, *e.g.*, the Holstein-Friesians, such a formative period of considerable inbreeding must have been several generations prior to the birth of the animals which were imported into the United States, if such a period existed at all. It remains arguable that such a pedigree bottleneck as existed in the Shorthorn breed when most of the ancestry of the breed was in the herds of the two Colling brothers who both built their herds largely by the intensive use of the one bull, Favourite, may have been almost a unique event in the origin of breeds, instead of having been the usual situation. In most breeds the recording of individual pedigrees did not begin until after the breed had already come

to be considered by its owners as a valuable group worth keeping free from admixture with outside blood.

The last column of Table 1 shows that the breeds generally showed only a moderate tendency to subdivide into partially isolated subgroups, although in many breeds there was at least a little of this. Such subdivision has not been the primary cause of the increase in inbreeding, although it may have increased that to some extent by making families more distinct from each other than they would otherwise have been and by thus putting the economic competition and selection more on a family basis and less on an individual basis.

Discussion

It is clear from Table 1 that the random changes in gene frequency in the pure breeds of livestock are rather large. Which breeding practices are mainly responsible for this are not wholly clear except that subdivision into partly non-interbreeding groups (whether by distance alone or because some breeders deliberately practice some form of inbreeding) will account for only a small part of it.

Presumably the general striving to get sons and grandsons of the currently most famous sires is a major factor, but no way is apparent to appraise separately (1) the effects of that and (2) the effects of the stratification of herds into breeder's herds and multiplier's herds, and (3) the chance elimination of ancestors even two or more generations after they themselves are dead. Some examples showing how important each of these can be will be presented next. These examples were picked because they were unusually extreme among those known to the writer, although probably not the most extreme which ever did occur.

In the Hereford breed of cattle in the United States as of 1930 more than one seventh of the genes came through the bull, Beau Brummel; about half that many more came

from his sire through Beau Brummel's brothers and sisters; and about one ninth more came from his two grandsires through his aunts and uncles.

The most extreme example yet found of one animal providing a large fraction of the genes of a whole breed comes from the formative period of the Shorthorn breed of cattle in England. More than half of the randomly traced ancestral lines in that breed go through one bull, Favourite, who thus may be said to have sired the whole breed.

Nearly every breed yet studied shows cases in which 5 per cent. or more of the genes have come through each of several ancestors, plus or minus (of course) whatever subsequent selection may have done to make the actual situation different from what the pedigree structure of the breed indicates.

In selecting breeding animals the emphasis is naturally on the males since far fewer of them than of the females need be saved for breeding. The possibilities for really intense selection are therefore greater among the males. Because the males are fewer, chance in their choice can play a larger part in changing the gene frequency of the whole breed than can chance in the choice of females. Yet each animal has as many female as male ancestors in each generation. It is no denial of the importance of choosing a good sire to admit that about half of all the genes which he transmits are genes which came to him through his dam! Examples of females which furnished a large fraction of the genes of a breed can be found, although such females are not as numerous as equally prominent males. About one eighth of the lines randomly traced in the pedigrees of the present Holstein-Friesian breed of dairy cattle in the United States lead to one cow, De Kol 2nd. That she achieved this importance mostly through the extensive use of her many sons (rather than her daughters) is beside the point. Each gene in her had to have a frequency of zero, .5 or 1.0. The extensive use of

her descendants can easily have altered by .03 or more[5] the frequency of most genes in the breed which did not already have frequencies near to zero or 1.0. This would have happened even for such genes as were neutral or even slightly undesirable as far as selection for or against them is concerned. The case of De Kol 2nd will illustrate how large the changes in gene frequency due to preference for one female ancestor can be, even in a species as unprolific as cattle.

An example of how the stratification of a flock into a stud flock and a commercial flock can permit gene frequency to change widely is afforded by some data from a large poultry flock[6] which adds each year about 10,000 female and 800 male replacements, all of which come from the pedigree or stud section of its own stock. All the replacements hatched in 1943 were sired by only 65 males. But 49 of these 65 were in seven groups of full brothers, and two of these groups were half-sib to each other. Two of the remaining 16 sires were each a sire of one of these seven sets of full brothers. Several others were half brothers or uncles or other close relatives. Thus well over a third of the genes in these 1943 replacements (nearly 11,000 birds) came from only 13 individuals[7] as sires or parental grandparents. Chance in the choice of these 13 animals could easily have raised or lowered gene frequency in so large a general flock as this by .04 or more for any gene which didn't have a frequency already close to zero or to 1.0 in the flock from which these 13 were a sample. Had 1,000 individuals—instead of 13—provided

[5] The variance of the chance changes in gene frequency because $1/x$ of the genes in the breed came from one individual—which itself was the sum of only two gametes from the preceding generation—would be approximately $\frac{q(1-q)}{2x^2}$, which gives a standard deviation of .04 when q is near .5 and x is eight.

[6] Kimber Poultry Breeding Farm, Niles, California.

[7] The 13 parents of 49 of the 65 sires plus the two sires which were themselves included in the 13. 51/65 = .78 of the one half of their inheritance which the replacements received from their sires.

the inheritance through the sires or paternal grandsires, selection would have been less intense, but the chance variations would have had more opportunity to cancel each other into insignificance.

As an example of what the elimination of ancestors may do to the pedigree structure of a population, see Stonaker's (1943) graphic presentation of how almost half the genes of the Aberdeen-Angus breed of cattle come from a small group of 14 ancestors, many of which are closely related to each other. As another example, Table 2 shows how few of the individuals which were chosen for breeding in an isolated population of swine actually had any descendants there several generations later.

TABLE 2

TOTAL NUMBER OF ANCESTORS WHICH CONTRIBUTED TO THE SCOUTMASTER HERD IN THE AUTUMN OF 1944

Year born	Female ancestors	Male ancestors
1930	12 (+2*)	4
1931	6	2
1932	1	3
1933	7	3
1934	2	2
1935	7	3
1936	6	4
1937	4	2
1938	5	3
1939	8	4
1940	4	3
1941	13	4
1942	12	4
1943	27	5
1944	41	7

* These two were actually born in 1929 but were purchased in 1930 when the experiment was started.

The data came from a Poland-China herd established at the Iowa Agricultural Experiment Station in 1930 and replenished entirely from within itself ever since. The breeding plan was to use four males and from 40 to 50 females each year. In the early years progeny testing was emphasized. Consequently many of the sows and some of the boars were used again in a second year and occasionally even in a third and fourth year. In recent years emphasis has swung more to sib testing with the result that few of the boars and only about a fifth of

the sows are kept for a second year. The only conscious attention paid to pedigree when selecting the young boars and gilts for use was that much attention was given to the productivity of the dam and some attention to the phenotypic merit of litter mates. This naturally led to a strong correlation between the fates of maternal sibs. Accidents during the breeding season, the occasional maintenance of a separate group in quarantine for health reasons, etc., sometimes made necessary the use of boars or sows which otherwise would only have been held in reserve. Hence more than four young boars and more than 40 young gilts were actually used in some years. Table 2 shows for the whole group which entered the breeding pens in the fall of 1944 the total number of different ancestors which were born in each preceding year since the date when an absolute pedigree barrier was erected around this herd.

The elimination of potential ancestors was rapid in the first two to four years after they themselves entered the breeding herd. Many of the 1944 animals will have no offspring enter the herd in 1945. Many of the 1943 animals and some of the 1942 animals which have 1944 offspring in the herd will have no descendants saved in 1945. The elimination of animals as potential ancestors continues rapidly for at least three or four years after they first enter the herd and more slowly for some time longer. One sow born in 1932 still had descendants in 1942 but none in 1944. Detailed examination of the herd pedigree indicates that six of the 13 females born in 1941 are still so precariously represented that they are likely to be eliminated in the next two or three years. The other seven are in the pedigrees of so many of the 1944 animals that their complete elimination as ancestors appears highly improbable. As the population pedigree is traced farther and farther back, a date is reached at which each ancestor is in the pedigree of every living individual. Further elimination of ancestors from the pedigree is

then impossible as long as the population remains isolated. Even though an ancestor remains in the pedigree of the population, all the genes which it carried can be eliminated because of the Mendelian segregation processes whereby a grandson or granddaughter or more remote descendant could—and for many genes certainly would—receive nothing from this ancestor. But whether that happened in a particular case can not be learned from the pedigree alone.

The pedigree structure of pure breeds or of races of man or of geographical subgroups of other animals in nature behaves in some degree like this mildly inbred line. The chief difference is probably that the time base to which pedigrees must be traced before all members of the same race have nearly the same network of ancestry is usually more generations into the past than the three or four generations which suffice in this small population of swine. Also in wild populations the isolation will often be less complete than in this herd and there will be some addition of ancestors when immigrants are absorbed. Presumably this pedigree isolation is in most cases the primary cause for one breed or local race becoming distinct from other such nearby groups in the first place, although selection gradients being different from breed to breed or from one locality to another doubtless play a part in some cases.

Detailed examination of the pedigree map for this herd of swine shows that the entire inheritance of the herd in 1944 came through 34 gametes which either produced the animals born in 1941 or were produced by them. Still narrower bottlenecks existed earlier. In or about 1939 the entire inheritance was in 22 gametes; in or about 1938 all of it came through 22 gametes; in or about 1935 through 29 gametes, in or about 1931 through 28 gametes; out of 1930 through 22 gametes. Now the chance presence or absence of a gene from even one of these gametes would shift the frequency of that gene in this herd up or

down by about .04, even if all these gametes went into individuals which had a chance to contribute equally to the next generation. Chance might well alter gene frequency by three or four times that much from one generation to the next for any gene with an intermediate frequency (*e.g.*, .2 to .8) in the population of parents from which these few gametes were a sample.

Chance shifts of gene frequency in a whole breed of livestock in one generation are doubtless smaller than in this mildly inbred line. However, a ratio as extreme as that between the 22 to 30 gametes actually found in these pedigree bottlenecks and the 90 to 100 which constituted the animals placed in the breeding pens may well exist in whole breeds where little or none of what we ordinarily call inbreeding is being practiced. This elimination of potential ancestors may well make the effective number in the breeding population only a small fraction of the total number which actually reach breeding age. The third column of figures in Table 1 shows that the effective number actually is small. Since most of these breeds had in all recent years many thousands of living members, the disparity between effective number and census number evidently is extreme.

Summary

Chance deviations in gene frequency from one generation to the next, as a result of Mendelian sampling and the elimination of potential parents, are important in the pure breeds of livestock. Standard deviations of the order of .02 to .05 for gene frequencies near .5 seem to be about the usual magnitude of these chance changes in one generation.

The major cause for these changes being so much larger than would be required automatically by the finite size of the population seems to be inequality in the number of gametes which the various potential parents actually contribute to the next generation.

Attention to family and pedigree, plus the economic incidents which happen to whole herds, probably emphasize this more in livestock than is usual in most species in nature, although this statement about pure-bred livestock as compared with wild species is an expression of faith which might be severely shaken if we knew more about the actual pedigrees of wild organisms!

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OBSERVATIONS AND EXPERIMENTS ON THE CAVE FISH PIMELODELLA KRONEI AND ITS RELATIVES

DR. C. PAVAN

UNIVERSITY OF SÃO PAULO, BRAZIL, AND COLUMBIA UNIVERSITY, NEW YORK

INTRODUCTION

RUDIMENTATION of some organs combined with adaptive modification of others is common in cave animals. The origin of these peculiar forms of life is one of the interesting problems of the theory of evolution. In the days when Lamarckism was in vogue, cave animals were cited as spectacular evidence of the inheritance of the results of use and disuse of organs. When this explanation was almost universally abandoned, the rudimentation in cave animals of organs that are very important in their free-living relatives became an example of the effects of mutation pressure unopposed by natural selection (Wright, 1929; Emerson, 1938; Dobzhansky, 1941). Yet, it can hardly be denied that the evidence, particularly experimental evidence, is not quite sufficient to prove the validity of the modern view either, and that further data, especially on cave animals showing transitions toward, or living near to, their presumed free-living ancestors are much to be desired. Breder (1942, 1943) and Breder and Gresser (1941) have recently published important work on the fish *Anoptichthys* from a cave in Mexico. A report on the blind fish *Typhlobagrus kronei* Miranda Ribeiro, living in certain caves in the state of São Paulo, Brazil, is presented here. The *Typhlobagrus* case is in some respects parallel, and in others complementary, to that of *Anoptichthys*.

This work has been executed under the direction of Professor André Dreyfus, of the University of São Paulo, to whom the author owes a great debt of gratitude. Encouragement, advice and help in the prepara-

tion of the English manuscript have been received from Professor Th. Dobzhansky.

The Caves

T. kronei is known to occur in two limestone caves in the township of Iporanga in the southwestern part of the state of São Paulo, Brazil. The location of the caves, known respectively as Areias and Bombas, is shown in

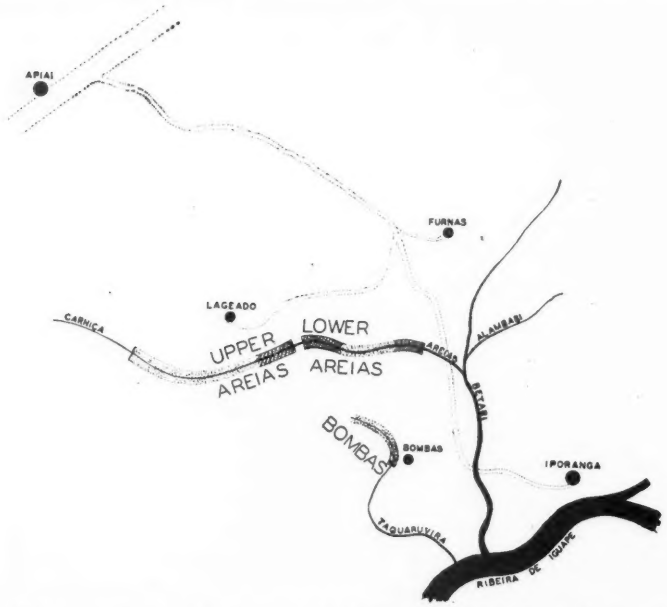

Fig. 1. A map showing the approximate locations of the caves and streams in which *P. transitoria* and *P. kronei* are found.

Fig. 1. No detailed map of this region seems to exist, the one in Fig. 1 being drawn free hand without any pretension at being accurate as to scale.

The Areias cave is located in a small valley surrounded on all sides by low mountains. The river which flows through the cave arises in the jungle on the other side of the mountain under which the cave is situated. After cascading for several kilometers over a steep slope in the

jungle, the river disappears at the base of a vertical rocky wall, and reappears from the mouth of the Upper Areias cave. The stream in the jungle is called Corrego da Carniça, the one flowing through the cave Corrego de Areias.

Inside the known portion of the cave the stream bed is flat and the current fairly slow. Corrego de Areias flows on the ground surface for only 30 meters and disappears again into another cave called the Lower Areias cave. The latter cave pierces a steep hill and opens on the other side, giving rise to a 700 meters long stream, flowing into the right side of Rio Betari only about 30 meters below the confluence of Rio Betari with Rio Alambari. A survey was made of the distribution of *T. kronei* in various accessible parts of the stream system just outlined. Specimens were found only in the interior of the Upper Areias cave and just inside the upper entrance to the Lower Areias cave.

The Bombas cave is near the village of the same name. The stream emerging from Bombas, known as Taquaruvira, cascades down a steep slope for 10 to 12 kilometers, and falls into the river Ribeira de Iguape approximately 1,200 meters upstream from the confluence of the latter with Rio Betari. About 2 kilometers from its mouth the Taquaruvira stream forms a waterfall with a vertical drop of approximately 8 meters. This waterfall is presumably an absolute barrier for the passage of fish upstream. The only time when such a passage seems conceivable is during the season of floods, when the Ribeira de Iguape rises 6 to 8 meters above its normal level. However, the waterfall is located about 15 meters above the river level, thus making a communication improbable. The mouth of Bombas cave is too small to admit entrance of a man, so that the interior of the cave is not known. It is however, virtually certain that the Taquaruvira stream arises inside the cave, and its water while in the cave seems to carry no nutritive material. *T. kronei* emerges from the cave nightly to feed in the part of the stream at most 50 meters below the mouth of the cave.

Morphology of the Cave and the Free-living Fish

Miranda Ribeiro (1905) described a species of catfish *Pimelodella transitoria* from Rio Alambari, state of São Paulo, Brazil. According to Eigenmann (1917) the distribution of this species extends from Rio de Janeiro to Baia de Paranaguá, state of Paraná. Our collection of this common form came from the neighborhood of the caves, namely, from the Rio Ribeira de Iguape, Rio Betari, Rio Alambari and Rio Areias. In the Areias River a single specimen has been caught well inside the upper Areias cave, and about 40 specimens outside the cave in the part of the stream flowing into Rio Betari. None were found in the Taquaruvira stream issuing from the Bombas cave. This species occurs by preference in deep and quiet pools in the rivers in which it lives. In daytime it hides under stones and plant roots, and becomes active at night during which time it can easily be caught by angling with earthworm bait. Only on rainy days and when the rivers are in a state of flood can individuals of this species be found procuring food during the hours of light.

Two years later, Miranda Ribeiro (1907) described specimens of catfish collected by Ricardo Krone in the Upper Areias cave, and classified them as representing a new genus and species—*Typhlobagrus kronei.* In a later paper (1911) Miranda Ribeiro pointed out the probable descent of *T. kronei* from *P. transitoria,* without, however, recognizing their generic identity. The same course was followed by Eigenmann (1917), except that the latter author regarded *P. transitoria* to be only a variety of the widely distributed *Pimelodella lateristriga* Müller and Troschel.

A careful comparative study of the morphological characteristics of 71 specimens of *P. transitoria* and 65 specimens of *T. kronei* from Areias cave and 64 specimens from Bombas cave has been made. A detailed description of these fishes will be published elsewhere. Suffice it to be stated here that they are alike within the

limits of statistical errors in all measurable and qualitative characters, except the following ones. All the individuals of *P. transitoria* have completely developed eyes. All the 114 specimens of *T. kronei* collected by the writer in 1942–1944 proved to lack any anatomically visible vestiges of eyes or of optic nerves. However, among the 15 specimens collected in the Upper Areias cave by Dr. Othon Leonardos in 1933 and kindly pre-

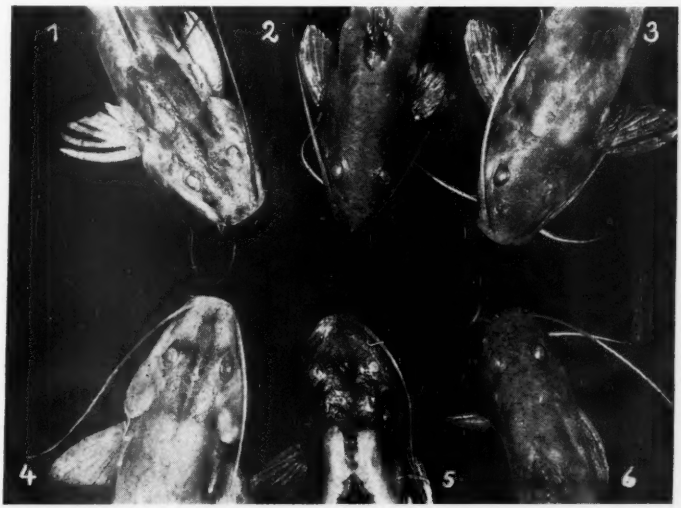

FIG. 2. Types of head shape in *P. transitoria*. Type A—specimens Nos. 1, 3, and 4. Type B—Nos. 2 and 6. Type C—No. 5.

sented by him to the writer for investigations there were 2 specimens with rudimentary eyes. One of the latter had eyes about half normal in diameter under the epidermis on both sides of the head; the presence of optical nerves was ascertained by dissection. The second had a diminutive eyeball on one side of the head only; unfortunately, no dissection was made. Miranda Ribeiro (1907) likewise found one fish among his 35 specimens which had, according to his description, an almost normal eye on one side of the head and none on the other.

P. transitoria shows a considerable variation in the form of the head. The following three types connected by intermediates can be distinguished. Type A (Fig. 2, specimens Nos. 1, 3 and 4)—head shaped like a truncated cone. Type B (Fig. 2, specimens Nos. 2 and 6)—like the preceding but the sides (as seen from above) more or less curved. Type C (Fig. 2, specimen No. 5) with a pointed snout. In the Areias population of *T. kronei* there occur

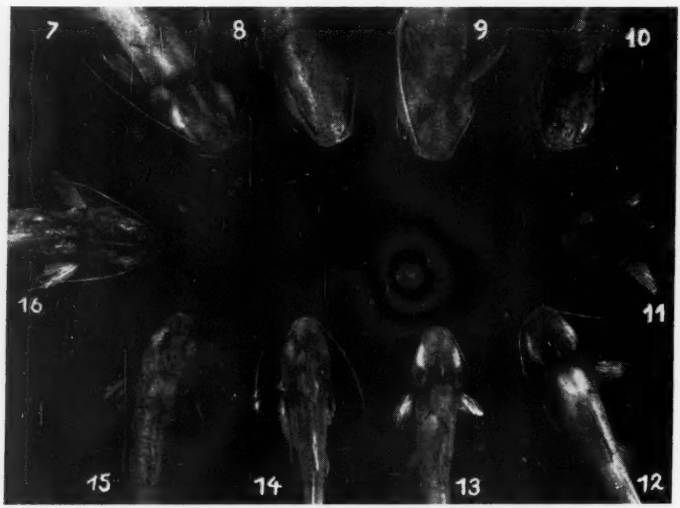

FIG. 3. Types of head shape in the Areias population of *P. kronei*. Type A—specimens Nos. 7, 9, and 10. Type B—Nos. 14 and 15. Type C—Nos. 11 and 16. Type D—No. 8.

Type A (Fig. 3, Nos. 7, 9 and 10), B (Nos. 14 and 15), C (Nos. 11 and 16) and a single specimen of Type D (Fig. 3, No. 8,) with an abruptly truncated head. The Bombas population of *T. kronei* has heads of Type E (Fig. 4, Nos. 17, 22 and 23) compressed and flattened from above, and Type F (Fig. 4, Nos. 18, 21 and 24) which can be described as intermediate between Types A and E. The incidence of these types in the three populations is shown in Table 1.

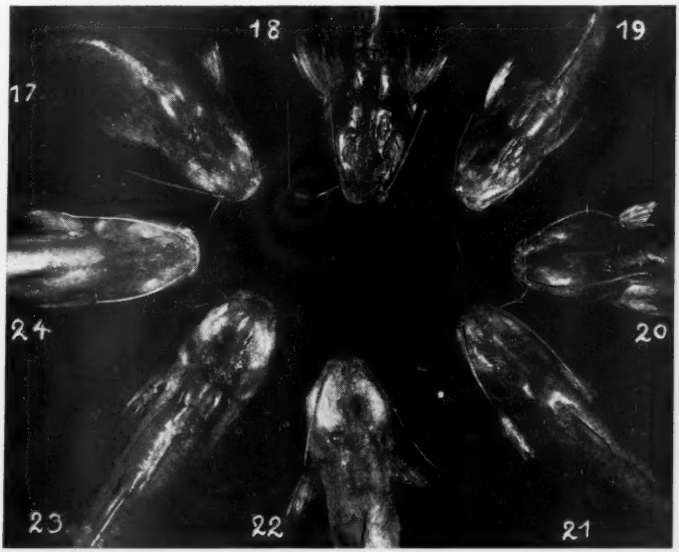

FIG. 4. Types of head shape in the Bombas population of *P. kronei*. Type E—specimens Nos. 17, 22, and 23. Type F—Nos. 18, 21, and 24. Specimens Nos. 19 and 20—intermediates between the types E and F.

It appears, then, that at least as far as the head shape is concerned, the Areias population of *T. kronei* resembles the free-living *P. transitoria* more than it does the Bombas population of *T. kronei*.

The pectoral fin contains a solid ray which carries the following system of teeth (Fig. 6): a row on the posterior margin, the middle teeth of the row being longer than the marginal ones; a row of small reversed teeth on the dorsal part of the anterior margin; a row of teeth on the

TABLE 1

THE INCIDENCE OF VARIOUS TYPES OF THE HEAD SHAPE IN THE FREE LIVING P. TRANSITORIA AND IN THE TWO CAVE POPULATIONS OF T. KRONEI

	Head shape						
	A	B	C	D	E	F	Total
P. transitoria	20	38	6	..	..	..	64
T. kronei, Areias	36	17	6	1	..	..	60
T. kronei, Bombas	..	..	..	..	47	17	64

anterior margin which are smaller in size than those on the posterior and uniform in size. The teeth on the posterior and the reversed ones on the anterior margin are alike in *P. transitoria* and in *T. kronei,* or perhaps the reversed teeth are slightly larger in the Bombas population of the latter. The small teeth on the anterior

FIG. 5. A specimen of *P. transitoria* (center) between two specimens of *P. kronei* from Areias cave.

margin are variable. Among the 71 individuals of *P. transitoria* investigated in the respect, 52 had more than 15, and 19 individuals from 15 to 0 such teeth. Among the 65 *T. kronei* from Areias, the corresponding numbers were 48 and 17. In the Bombas population most of the 64 studied individuals had 4 to 5 teeth, and none had 15 or more.

T. kronei as well as *P. transitoria* have six barbels,

four on the mandible and two on the maxilla. The barbels are important as tactile and gustatory sense organs. They are capable of regeneration when cut; an individual of *T. kronei* 130 mm long has regenerated a section of the maxillar barbel 46 mm long in less than 6 months. The histological structure of the barbels in our fishes is like that described by Olmested (1920) in *Ameiurus nebulosus*. The maxillary pair of barbels shows interesting variations. In *P. transitoria* the length of these barbels varies to such an extent that the end of the barbel may reach in some individuals only to the tip of the pectoral fin when closed, while in other individuals it reaches as far as the base of the anal fin. In the Areias population of *T. kronei* the barbels reach at most to the apex of the ventral fin; in the Bombas population they are shorter still, reaching at most to the base of the ventral fin.

FIG. 6. A pectoral spine of *P. transitoria*.

The coloration of most catfishes changes rapidly depending on the color of the background in which the fishes live. As will be shown below, *T. kronei* lacks this adjustment, which is strikingly apparent in *P. transitoria*. On the other hand, when exposed to light *T. kronei* becomes very gradually darker than it was without light. Whether or not this slow type of changes occurs also in *P. transitoria* is not known. For these reasons a study of the color variation in *P. transitoria* is difficult, and no exact classification of the available material was undertaken. All we can say is that individuals of that species when living on dark background vary from gray to dark gray. The coloration of *T. kronei* was recorded immediately after these animals were brought to the laboratory in São Paulo and before they were exposed to light. The average coloration proved to be darker in the Bombas than in Areias population. This is probably due to the fact already mentioned above, namely, that the in-

habitants of Bombas leave the cave nightly for feeding, and in all likelihood live near the cave entrance, and therefore are exposed to some light. The inhabitants of Areias live in permanent darkness, and form little pigment. The difference is apparent from inspection of Table 2.

The variation of the dark strip on the lateral line parallels that of the general body pigmentation. The strip is absent in the light individuals from Areias and Bombas, but present in the more heavily pigmented in-

TABLE 2

COLORATION OF P. TRANSITORIA AND T. KRONEI

	Gray to dark gray	Light gray	Grayish yellow	No visible pigment	Total
P. transitoria	71	..	..	..	71
T. kronei, Areias ...	..	12	21	9	42
T. kronei, Bombas ..	15	37	6	..	58

dividuals. *P. transitoria* is always pigmented, although its coloration is, as shown below, subject to much variation.

BEHAVIOR OF P. TRANSITORIA

As stated above, *P. transitoria* is a nocturnal animal in its natural habitats. Its food consists, as shown by studies on the intestinal contents, of insect larvae and adult insects, as well as of small lamellibranch and gasteropod molluscs. Many individuals dissected had the intestinal canal empty.

In our aquaria *P. transitoria* showed a sharp negative phototropism, spending all days hidden under stones or in the midst of vegetation. If disturbed, the fish darts across the aquarium seeking a place to hide. Placed in an aquarium fully accessible to light and lacking protection where they may hide, the fishes become greatly agitated and attempt to squeeze themselves as tightly as possible in one of the corners of the aquarium. Pieces of meat were used as food for the captive specimens. Food must be given at night, since during the daytime the fish does not take the meal offered to it. At night, the fish

comes to the food only after the electric light used to facilitate the operation is extinguished. A light flash causes them to run for a dark place.

The time necessary for color changes on different backgrounds varies somewhat from individual to individual, and moreover the darkening reaction is much faster than the lightening. If two individuals with about the same dark gray color are placed in aquaria with black and with white walls, respectively, the animal in the dark aquarium becomes protectively colored in a few minutes. The fish in the white aquarium is greatly agitated while its pigment cells are contracting. In about 2 hours the animal becomes yellowish pink, sometimes with irregular dark spots resembling those seen in some *T. kronei* living in light aquaria for a long time. The tips of the fins are most likely to preserve pigmentation. These spots disappear completely in about six hours. If such bleached fishes are returned to an aquarium with black walls, they become rather darkly pigmented in less than ten minutes. This suggests that in Pimelodella, like in *Mustelus canis* studied by Parker (1934), the expansion and contraction of the pigment cells are governed by two different mechanisms. However, in *P. transitoria* injections of pituitary extracts resulted in contraction of the pigment, while in *M. canis* they result in expansion.

Some specimens of *P. transitoria* were made blind by destroying their eyes by injection of india ink into the eye cavity. The blinded animals became dark regardless of the color they had before the operation. The loss of the reaction to the color of the background did not result in a loss of the tendency to hide under stones or among plants. Nevertheless, some blinded fish remained stationary in exposed parts of the aquarium. Fish with only one eye destroyed when living in white aquarium became grayish-pink. In the same aquarium but without light they contracted the pigment cells completely.

Behavior of T. kronei

As shown above, the free-living fish, *P. transitoria,* has

a sharp diurnal rhythm, with activity confined to the dark and reposes to the light hours. By contrast, the cave form, *T. kronei,* shows either an uninterrupted activity regardless of the light conditions, or else a reaction to light much less pronounced than that in its free-living relatives.

About thirty individuals of *T. kronei,* 24 from Areias and 6 from Bombas cave, were kept in the laboratory in a cement aquarium 1½ meters square for several months, some of them for more than a year. These animals are actively swimming in the aquarium at all times, exhibiting a somewhat greater activity in late afternoon, during evenings, mornings and at night. They accept food offered to them at any time and regardless of the light conditions. The amount of food given must be controlled, since the animals are inclined to gorge themselves to death. This has repeatedly been the cause of loss of experimental fish, particularly at the beginning of the captivity in the aquarium. Only some individuals exhibited a slightly different behavior, by seeking a corner or a place under a stone in which to spend a greater part of the day, and by confining their foraging excursions to the night hours. It must, however, be emphasized that the negative phototropism of even these rather exceptional individuals of *T. kronei* is much less intense than that of all known *P. transitoria.* The 100 candles light bulb which was used at night in working with the fish produces no effect whatever on any of *T. kronei.* Only the direct sunlight causes *T. kronei* to withdraw into shade. A pink sheet placed in various parts of the aquarium caused the fishes to assemble under it until the afternoon hours when light intensity diminished (the experimental aquarium stands on an outdoor balcony).

In nature, the behavior of *T. kronei* inhabiting the Areias cave must be different from that of the inhabitants of Bombas cave. As stated above, the stream arising in Bombas cave contains no nourishment, thus neces-

sitating the fish making foraging excursions outside. Such excursions are made exclusively during the night. No specimens were ever caught at the cave entrance before darkness. In Areias cave, the fishes were never observed by us to approach the mouth of the cave at any hours, although Dr. Othon Leonardos informs us that he did trap some of them near the entrance in 1933. The food of *T. kronei* in both caves was shown by dissection to be the same as in *P. transitoria.* The intestine of one of the individuals contained approximately 50 small gastropod mollusks; another individual contained 3 amphipod crustaceans 4–5 mm. long; several individuals had remains of insects; one intestine contained many starch granules as though coming from some seeds; and finally some dissected specimens had the intestines empty.

Color Changes in T. kronei

T. kronei does not change its pigmentation rapidly regardless of the color of the walls in the aquarium in which it is placed. In this respect the situation in *T. kronei* stands in a contrast with that in *P. transitoria.* However, *T. kronei,* or at least some individuals of this form, display changes in their pigmentation which take place under the influence of light, but which require weeks or months of exposure to become noticeable.

The color of *T. kronei* collected in the caves varies from a complete absence of visible pigment up to gray. It may be noted that among the nine individuals which show no pigment when inspected with naked eye five individuals had numerous light brown chromatophores visible with the aid of a binocular microscope at a magnification of 48 times. Other four individuals showed no pigment cells under that magnification either. When living in the aquarium exposed to daylight illumination the initially weakly pigmented fishes have after some weeks gradually developed darker and darker pigmentation. The individuals in which this darkening process was most intense have become after some months black-

ish gray in color, as dark as the darkest known individuals of the free-living *P. transitoria.* Unfortunately, none of the above mentioned four completely unpigmented individuals survived long enough to be sure that they could not develop a pigmentation.

A fact of much interest is that the capacity of developing a pigmentation after exposure to light varies greatly from individual to individual of *T. kronei.*

Among 13 individuals that lived in the aquarium exposed to light for more than 6 months the coloration varied from a dirty yellowish pink up to a blackish gray. Two of the lighter individuals were used for the following experiment. One was placed for three months in a box submerged in the general aquarium but so constructed that little or no light penetrated to the fish. The other individual was left in the light. After three months, the two individuals were very nearly alike in coloration, the one which lived in the open having only very slightly more pigment than the other. A similar experiment was made with two of the darkly pigmented individuals; here no change at all was noted. The pigmentation once formed in the light either does not regress or does so only very slowly in the dark. It seems, furthermore, that the process of the development of the pigment is about completed in less than six months, and longer light exposures result in no further darkening.

The pigment developed in *T. kronei* as a result of relatively prolonged exposures to light does not acquire the ability of rapid contraction and expansion in accordance with the coloration of the background on which the fish lives. In this respect this pigmentation behaves very differently from the apparently similar pigmentation of *P. transitoria.* Of course, it must be noted that no pigment contraction or expansion is observed in *P. transitoria* with destroyed eyes either. Some of our darkened individuals of *T. kronei* were kept in an aquarium with white walls for two weeks without any alteration of the color.

Influence of Pituitary Extracts on T. kronei

Some individuals of *T. kronei* in our aquaria behaved as though in a breeding period. Two individuals swim side by side, their movements being in close harmony, push each other by their heads in the abdomens, strike each other gently by the spiny fin rays, bite each other's barbels, etc. This behavior lasted for about an hour at a time, often several times in the same day. When no eggs were deposited it was decided to attempt a stimulation of reproduction by means of injections of pituitary gland extracts. The attempt failed to bring about the production of offspring. It may, however, be reported that pituitary extracts have pronounced effects on the pigment cells.

Through the courtesy of Dr. Pedro de Azevedo fish pituitaries preserved in absolute alcohol were obtained. Each gland, about 1 mm^3. in volume, was ground in 2 cc. of Ringer's solution, and 0.25 cc. of this liquid was injected at a time into a fish. The pigment around the point of injection visibly disappears within a minute. In about 10 minutes after this injection the animal is spotted. Sometimes the body is light with dark spots, resembling those encountered in some individuals of *P. transitoria* bleached in aquaria with white walls. A little later the spots disappear, the animal becomes visibly devoid of pigment, and remains so for several hours. Traces of the pigment contraction are still noticeable in specimens injected as many as 30 hours before. Finally the coloration which the animal had before the injection is entirely restored. The timing of the processes of depigmentation and restoration of color depends upon the amount of the pituitary extract injected in relation to the size of the body, as well as upon the amount of the pigment present. Injection of Ringer's solution without the pituitary was made as a control; no effect on the pigmentation resulted.

Discussion

Typhlobagrus kronei, this typical cave dweller, blind

and frequently devoid of pigmentation, exists in an immediate geographical proximity to the free-living form, *Pimelodella transitoria,* from which it was, beyond reasonable doubt, phylogenetically derived. In this respect, the situation described above is analogous to that of *Anoptichthys jordani* and *Astyanax mexicanus* studied by Breder and his collaborators. In both instances the cave forms have been described as belonging not only to different species but to different genera from their free-living ancestors. Yet, the structural similarities between *T. kronei* and *P. transitoria* are great enough to make the generic separation hardly tenable, and the same probably holds for Anoptichthys and Astyanax. In fact, unless one adheres to a purely morphological species concept, the possibility that the cave and the free-living forms are members of the same species must be considered.

The behavioral differences between *T. kronei* and *P. transitoria,* particularly the differences in their reactions to light, would very likely make them incompatible at mating period, thus engendering sexual isolation. Breder and Gresser (1941) observed that in *A. jordani* and *A. mexicanus* the blind animals are often killed by these with vision. Unfortunately, we have not succeeded to breed either *T. kronei* or *P. transitoria* in captivity, and the above inference remains completely unverified. A more positive evidence of the existence of reproductive isolation, and hence of specific distinction, between *T. kronei* and *P. transitoria* is the finding of a single individual of the latter in the Upper Areias cave, in which the former is abundant (see above). Unless the presence of *P. transitoria* in the case is a rare accident, that species and *T. kronei* must be either reproductively isolated or else they would interbreed. Interbreeding would produce gene recombinations that would almost certainly show phenotypes intermediate between the parents. No such intermediates are known, unless the occasional individuals with rudimentary eyes be considered as such. Even if *P. transitoria* enters the cave only rarely, it is common enough

in the river just outside the cave to furnish opportunities for meetings with *T. kronei* at the entrance. Over long periods of time such meetings would probably be frequent enough to cause, in the absence of reproductive isolation, some interbreeding, gene exchange, and the origin of intermediates. The view that the cave and the free-living forms are to be regarded as closely related but distinct species seems at present most reasonable. It is hereby proposed to refer to these fishes as *Pimelodella transitoria* Miranda Ribeiro and *Pimelodella kronei* Miranda Ribeiro.

The morphological and physiological differences found between the populations of *P. kronei* from Areias and Bombas caves are remarkable. In both caves *P. kronei* differ from *P. transitoria* in the absence of eyes and a reduction of the pigmentation. Apart from this, the Areias population may differ from *P. transitoria* only in a slightly larger body size (the observed difference is not statistically significant). On the other hand, the structural differences between Bombas population and both the Areias *P. kronei* and the free-living *P. transitoria* are considerable. This raises the problem of the origin of the species population of *P. kronei.* Two possibilities present themselves. First, the Areias and Bombas populations may be descended from a common strain of a cave fish which had arisen in some one cave. Second, the Areias and Bombas populations may have developed independently from *P. transitoria,* the common characteristics of these populations (absence of the eyes, reduction of the pigmentation) being the result of a convergent process of loss of genes which maintain these characters in the free-living fishes. The data at hand do not permit to discriminate between these possibilities. The apparent isolation of Bombas cave and the internal uniformity of its population of *P. kronei* as compared to the greater variability of the Areias population of the same species suggest however that Bombas cave was originally populated by very few immigrants. The im-

migrants are geographically isolated both from the Areias *P. kronei* and from *P. transitoria.* This gave the opportunity for a divergence in the racial structural characters. On the contrary, the identity of the Areias *P. kronei* and *P. transitoria* (except for the eyes and pigmentation) suggest a lower degree of isolation and a relatively large genetically effective population.

Besides *P. transitoria* and *P. kronei,* two other fish species, namely *Rhamdia sp.* and *Rhamdioglanis sp.,* have been found in the Upper Areias cave. Of *Rhamdia sp.,* 12 individuals have been captured inside and 74 individuals outside the cave. Of *Rhamdioglanis sp.,* 10 individuals have been collected inside and none in the stream immediately outside the cave. No differences are apparent between *Rhamdia sp.* found inside and outside the cave. As to *Rhamdioglanis sp.,* it can merely be stated that no trace of organ reduction so frequent in cave animals is apparent in the specimens examined.

Summary

Pimelodella kronei is a fish species devoid of eyes and showing varying degrees of reduction of the pigmentation. It lives in the Areias and Bombas caves in the state of São Paulo, Brazil. Its very near relative and undoubted ancestor, *Pimelodella transitoria,* has well-developed eyes and pigmentation; it lives in many streams in the region in which the caves are located, and a single specimen of it has been collected inside the Areias cave. The Bombas population of *P. kronei* differs both from the Areias population of the same species and from the free-living *P. transitoria* in a series of structural characters, while the later two populations are similar except for the absence of the eyes and of the pigment in *P. kronei.* The Bombas and the Areias populations of *P. kronei* may have arisen either from a single blind and unpigmented population of cave dwellers, or else they may have developed convergently from a free-living ancestor.

Rapid contractions and expansions of the chromato-

phores can be induced in *P. transitoria* by placing it on light or on dark backgrounds. These reactions disappear following destruction of the eyes in *P. transitoria,* and they are absent in *P. kronei.* However, if exposed to the daylight for weeks or months in an aquarium, *P. kronei* gradually develops a dark pigmentation. The extent of this darkening is highly variable in different individuals; some individuals become as dark as the darkest *P. transitoria* while others change but little.

P. transitoria is sharply negatively phototropic. In nature as well as in captivity it swims actively during the night and rests in some hideout protected from light during the day. *P. kronei* is more tolerant or even indifferent to light. The behavior patterns are somewhat different in the populations which inhabit the Areias and the Bombas caves.

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NOTES ON THE BEHAVIOR OF THE GHOST CRAB

LORUS J. MILNE AND MARGERY J. MILNE
UNIVERSITY OF PENNSYLVANIA AND BEAVER COLLEGE

PROTECTIVE coloration and habits have interested most biologists. Few of the very many articles on these topics mention a remarkable example among the crustaceans. Certainly the ghost crab is large enough to be noticed easily, and is common from Atlantic City to Rio de Janeiro, on beaches where people vacation every summer and should have time for interesting observations. Yet there is only one good scientific report on the ghost crab of our Atlantic coastline, and this comes not from the American mainland but from the now abandoned laboratory of the Carnegie Institution of Washington on Loggerhead Key in the Dry Tortugas, beyond the tip of Florida. It would seem that the ghost crab has succeeded in concealing itself from zoologists as well as the general public.

The first observations made on this ghost crab were by the French naturalist, L. A. G. Bosc, on the coast of "Carolina" prior to 1802. Although Bosc grouped the fiddler crab (*Uca pugilator* (Latr.)) with the ghost crab (*Ocypoda quadrata* (Fab.)) in the genus *Ocypode* (the latter as *O. albicans* Bosc), his account shows clearly that he watched both of these land forms in the living state. Concerning the ghost crab, Bosc noted that "when they fear some danger, they save themselves by walking sidewise into their burrow with such rapidity that this naturalist was a long time observing them before forming an idea about the species of animal which was fleeing before him; it finally took all the speed of his horse to procure several specimens of them, again after several futile attempts. One knows well that an animal which is so difficult to catch can not serve commonly as nourishment; thus in Carolina no one makes any use of them. These

crabs are found also in the Antilles and in South America . . . . " Had Bosc lacked a horse and possessed some of the patience of his countryman, Fabre, he would have been able to observe the ghost crab in more detail. Procuring specimens by riding them down on horseback is certainly much more spectacular than laboriously digging them from their burrows by hand or with a shovel!

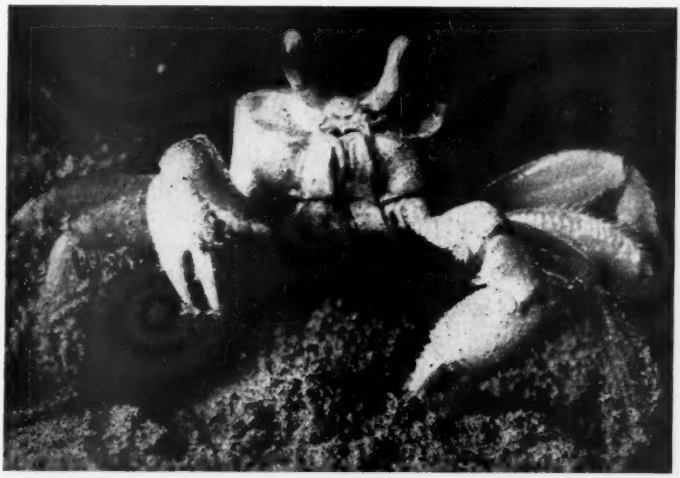

FIG. 1. The ghost crab (*Ocypoda quadrata*) on a New Jersey beach at night. When in danger or about to enter the burrow, the upright stalked eyes are dropped into the protective grooves seen along the front upper margin of the cephalothorax.

The genus *Ocypode* was erected by Fabricius (1798), for two of his own species (1793), our Atlantic ghost crab and the racing crab of the coasts of India and Malaya. *Ocypode* signifies "swift-of-foot," and Cott (1930) refers to the members of the genus as the "rabbits of the crustaceans." Our Atlantic coast species, *O. quadrata* (Fab.) has received a few lines of description in a number of technical and popular articles, and was presented to the lay public as a painting in the *National Geographic Magazine* (see Miner, 1937) and in *Natural History* (see Phillips, 1940). Except for Bosc's account and that from the

Tortugas Laboratory by Professor R. P. Cowles, the chief descriptions based upon original observation are those of Coues (1871), Verrill and Smith (1874) and Sawaya (1939). The Coues paper mentions that in the vicinity of Fort Macon, N. C., the young appear in April, are spotted and not yellowish like the adults, and that the crabs are put to no use except to afford excellent sport to boys and boyish men, who bait them with their canine companions! Sawaya's article (in Portuguese) compares the burrows of the ghost crab with those of the mole cricket, for sandy beaches near Sao Paulo, Brazil. Cowles (1908) based his studies on the very numerous specimens on Loggerhead Key, and observed them both on the open beach and with field glasses from the laboratory windows. He performed some interesting experiments to investigate their sense of smell and memory, as well as their otocysts, color changes and relation to water. His paper is informative and enjoyable reading.

Less extensive behavior studies have been made on three other species of *Ocypode*. The red racing crab, *O. ceratophthalma* (Fab.), has been observed by Cott (1930), and its stridulating habits and apparatus investigated by Alcock (1892) and Anderson (1894), while Hayasaki (1935) made plaster of Paris casts of its burrows and commented on the similarity between these and the "pipe rocks" of Cretaceous age. Takahasi (1935) investigated food and feeding habits in the same species along the coast of Formosa. Stebbing (1893) and Parenzan (1931) have also contributed notes on *O. ceratophthalma*. Along the Pacific coast of Central and South America occur *O. gaudichaudii* Milne-Edwards and Lucas (termed by Miss Jocelyn Crane the "painted ghost" crab), and *O. occidentalis* Stimpson. Miss Crane gave information on the immature stages, gross morphology and growth of both species in her 1940 and 1941 papers, and included behavior notes in these and her 1939 popular account of *O. gaudichaudii*.

The present writers have had opportunity to make

extended observations on *Ocypoda quadrata* near the northern limit of its distribution, at Townsend's Inlet, just north of Cape May on the New Jersey shore. They have been able also to maintain specimens in captivity for extended periods under almost domestic conditions. From data collected in these ways, further features of ghost crab behavior have been noted, as well as some difference in habits from those described in the literature. These notes and comments are largely supplementary to the excellent account by Professor Cowles (1908), and may involve variations of behavior patterns due to differences in habitat between the semi-tropical shores of Loggerhead Key and the more exposed, northern coast line of New Jersey.

Through the use of flashlamps and photoflash equipment, observations and records of ghost crab activity could be extended through the night when the animals are most active. By ten o'clock in the evening, the whole population of *Ocypode* is usually on the beach, and shows little fear of man or approaching lights. If a high tide has occurred during the day, the crabs are scattered along the line of beach drift which marks the farthest point reached by the waves, or between this line and the water. If the tide is high or almost so, the crabs are spaced at intervals along the limit of average wave advance. In all cases, each beached fish is the object of a single crab's attention, while other ocypodes investigate the damp sand for smaller food. In the latter activity, the two chelae ("pincers") are moved rapidly through the superficial layer of soil (two to three millimeters deep) and convey tiny particles to the mouthparts with rapidity and remarkable frequency. Takahasi (1935) and Crane (1939–41), covering the other three species of *Ocypode*, mention immature specimens taking sand into the mouth and straining it of microorganisms, the dried sand being formed into pellets which are ejected almost once a second. No such behavior was noted in the Cape May ghost crabs, and no pellets were found to evidence its existence.

Size variation is easy to study at night when all the ocypodes are near the water and may be approached without alarming them. In July at Townsend's Inlet, specimens ranged from quite small to very large, with most of the individuals of intermediate dimensions. The largest and smallest collected on one night measured 48 mm and 16 mm across the cephalothorax (span of outstretched ambulatory legs being from 232 mm to 73 mm). Males are much more numerous than females, the ratio being close to 3 : 1. In July on the New Jersey coast some females are still carrying eggs. Such gravid specimens are easy to recognize because their abdomens hang noticeably lower, being held away from the ventral surface of the cephalothorax by the rows of greenish yellow eggs glued to their swimmerets. Egg-bearing specimens differ even more strikingly in behavior. They wade out into the water much more freely than the males, and run along the bottom under water instead of clinging to the sand to wait for a wave to recede and leave them in air once more. While under water to a depth of a foot or less, they may be seen to wait until wave action has abated momentarily. Then comes a curious body movement not mentioned in the literature. The female opens her brood cavity slightly and with a sudden, continuous movement rocks her body completely upside down. The mouthparts, which normally face forward, are presented in rapid sequence first upward and finally posteriorly. The normal position is maintained by the ambulatory legs, although their bases undergo a marked rotation. Such a movement forces water through between the eggs with considerable violence. It was apparent that this body somersault took place only when the water was for the moment both calm and relatively free of suspended sand particles. Recovery to the normal position was equally rapid and the interval between inversion and the return movement very short. While discussing the behavior of the gravid female it may be well to add that during daylight, such specimens are equally distinct from males in reaction to disturbance.

If dug from their burrows and unable to find another opening nearby, they race for the water and run out under the waves quite beyond reach. Males, on the other hand, must be driven into the water day or night, and stop in it within a few inches, scarcely resisting capture.

The same rotation of the body upon the legs is shown by both sexes on the beach if sand is flicked at them. Since the chelae normally hang downward, and may be extended in front of the animal without changing the body position, such are the normal defensive attitudes. But when attempting to reach an attacker from directly overhead (such as is simulated by a light shower of sand), the body is rotated on the legs until the under surface of the cephalothorax is exposed, its dorsum nearest the ground, and the nippers reach into the air to grasp for any foe.

The free-swimming zoea of the Atlantic coast ghost crab is still unknown. Presumably it is similar to the corresponding immature stage in *Ocypoda gaudichaudii* described and illustrated by Miss Crane (1940). The transition to a land animal is by way of a megalops stage, and this phase of the life history of *O. quadrata* has been collected as far north as Vineyard Sound, off Cape Cod, Massachusetts (Smith, 1873, 1880; Verrill and Smith, 1874; Miers, 1876). Presumably these specimens had been carried north in the Gulf Stream and failure to colonize the coasts of New England may be due to the colder winter weather there. The farthest northward extension of adult *O. quadrata* involves the beaches on the south shore of Long Island, N. Y. Adult ocypodes seem quite sensitive to cold but can stand heat and intense radiation as long as the gill chambers are moist. Specimens in captivity behaved as normally as could be expected even when brilliantly illuminated by hot photoflood lamps only a foot and a half away. On the other hand, ghost crabs died quickly when placed in the refrigerator at 12° C., although the crustacean "Hippa" (*Emerita talpoidea* (Say)) lived in the same refrigerator for over a week.

In summer, small ghost crabs are found only close to

the shore, and their burrows extend often to water level. These young crabs are thus less completely terrestrial than their elders. They commonly make their tunnels in the wet sand left by the retreating tide and must remain buried during the day even when again covered by water, since none are found in daylight at high tide. Somewhat larger specimens burrow in the hard-packed sand beyond the high-tide mark, while the adults tunnel the more remote parts of the beach, invading even the nearby dunes for this purpose. The passageways of these mature specimens are seldom deep enough to reach water, although the sand below the top few inches is damp enough to pack well. The writers have not been able to learn whether the young crabs are able to keep their burrows from caving in as does any hole dug in the wave-soaked beach.

During late October a trip to the New Jersey coastline disclosed considerable ghost crab activity on a warm day. Toward sunset the occupant of each burrow cleared the tunnel mouth ready to emerge for a night's foraging, but only a few specimens were found active on the beach. These few were far smaller than any seen during the summer, but specimens which came out after 10:00 P.M. (under an almost full moon) included all sizes. All burrows found were on the drier parts of the beach, with many in the steep sides of the dunes (*cf.* Phillips, 1940). The crabs are light enough and their walking legs sufficiently sharp that they can run up the sloping sand there with little trouble. However, the sand grains in most cases are stacked at close to their critical angle, so that crab tracks disappear in miniature land slides, one per print. Tunnels which were excavated by the writers were far deeper than any studied earlier in the year. Apparently *Ocypoda quadrata* hibernates in the terrestrial condition, in a wide variety of sizes and far below the surface of the beach. The presence of very small specimens suggests that megalops develop and come ashore throughout the summer months. An extended breeding season may also be indicated.

Burrow-making is primarily an occupation of daylight hours. The crabs cease their feeding on the wet beach toward dawn. Those working on a beached fish often burrow within an inch or so of the carcass. Young crabs tunnel into the wet sand. Older specimens range inland and either occupy old passageways or build new ones. In either instance, the early morning hours are occupied with

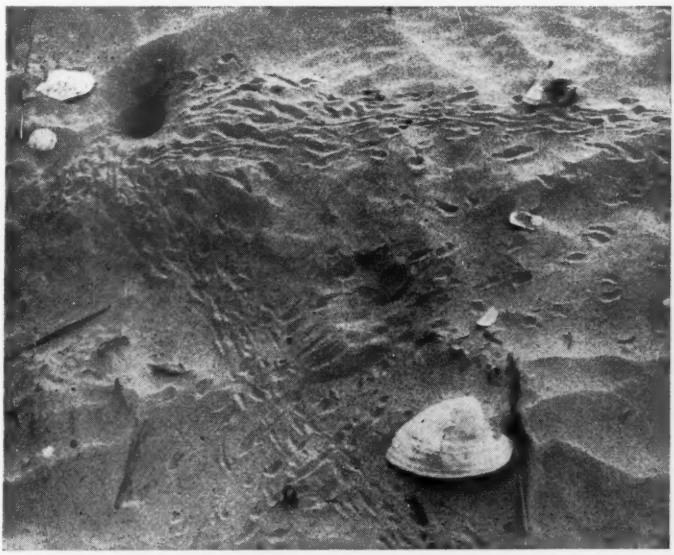

FIG. 2. Ghost crab tracks and burrow opening on the dry beach beyond any but the most violent storm waves. They may be found along Atlantic Coast beaches from the south shore of Long Island, N. Y., to Rio de Janeiro, including the West Indies and the shores of the Gulf of Mexico.

hauling up sand from below, extending or improving the tunnel. By sitting quietly beside the pile of damp earth brought up by a crab, a person can watch as the animal returns to the surface with further loads clutched in the curve of its walking legs. At first these burdens are brought to the top of the mound and tossed over its brim, or, if the crab suspects some danger, are left at the opening of the tunnel, where it first widens out to larger than

the crab's body. Later in the morning, the crabs level out the mounds. Often the earth is carried in rather small loads and spread over a fan-shaped area several feet in extent. The extremity of the fan is marked with little piles of still damp sand a few millimeters high, while the fan itself remains a maze of branching tracks. All the excavated sand is brought from one opening, even when the tunnel is U-shaped and the second aperture is fully formed. Toward noon, one or both openings may be closed from within by the crab, each plugged with a mass of damp earth which effectively hides the hole from all but the most diligent searching. If a slight wind is blowing on the beach, drifting loose sand in even small amounts, all traces of the crabs vanish from coasts where they are common.

When walking at their own selected speed along the beach, ghost crabs use all eight ambulatory legs and walk sidewise, often obliquely so. In loose sand their tracks show eight parallel rows of footprints. If pursued and unable to find a suitable hole into which to disappear, *Ocypode* runs rapidly, but unless the pursuer is very close, the same manner of locomotion is used and the tracks are not different from the normal pattern. If, however, the pursuer catches up with the crab, the added stimulus brings out a new running behavior not mentioned in the literature. The crab lifts its hindmost pair of legs clear of the ground and runs on six, making remarkably good progress in this way and seeming to gain in manoeverability. Under normal circumstances the direction of walking is usually toward the side with the smaller chela. When running on six legs, the crab darts in either sidewise direction (including oblique variations of these) with equal agility.

If surrounded or cornered in some way, adult ghost crabs present their nippers in front of them and may even attack a shoe or stick. Such attacks are usually very brief and serve to throw pursuers off balance long enough for the crab to find some new avenue for escape. A captured

crab with chelae held securely struggles violently, while its eyestalks are dropped into the protecting grooves along the front edge of the carapace. After a minute or so of firm handling, *Ocypode* becomes quiet except for spasmodic violent struggles. Under such circumstances the animal may raise its eyestalks, but it will do so much more promptly if the ambulatory legs are supported in approximately their normal position. An evidence of some acceptance of the *status quo* is the prompt wiping of the eyestalks with the maxillipeds, to clean them of

FIG. 3. In bright sunlight the ghost crab's shadow is more conspicuous than the animal itself. Tracks extend on both sides of this crab, made in efforts to sidle away from the photographer and his assistant.

sand or other debris. Thereafter the eyes remain erect. Usually the mouthparts themselves enclose sufficient water that bubbles may be seen forming and bursting with great rapidity over the tips of the maxillipeds.

Frequently when a ghost crab is running some distance in front of a pursuer, it stops for a moment. At such times it is very easy to lose sight of the animal, since it blends so well with the beach. In bright light, however, its shadow is so very black and so contrasty that the crab may be located again if the vicinity is searched carefully.

As mentioned briefly by Cowles, ghost crabs get rid of this shadow, too, if when running they find a small depression full of loose dry sand. The crab drops into the hollow and works its legs violently to sink body and appendages into the earth until only the upright eyestalks are exposed. The shadows cast by the eyes are not very noticeable even in the oblique light of early morning, and the crab will remain buried for a minute or more if the

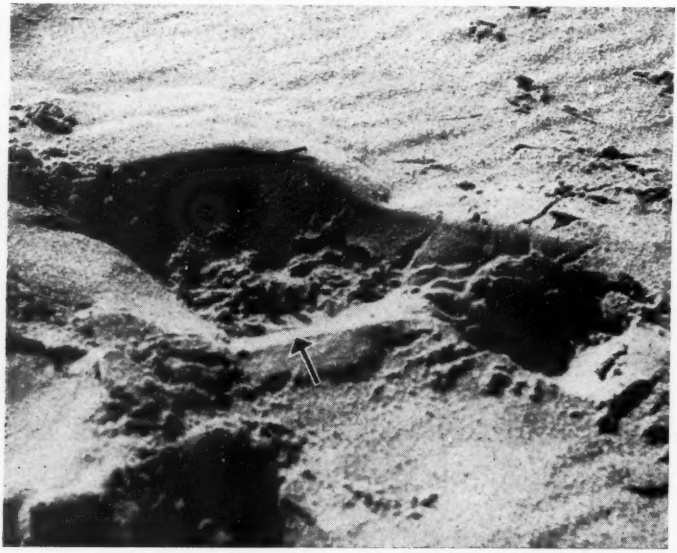

FIG. 4. If pursued in daylight, the ghost crab either disappears down a burrow opening or disposes of its tell-tale shadow by sinking body and legs into loose dry sand at the bottom of any little depression. Only the upright brown eyes (arrow) remain to cast shadows as the crab watches its pursuer go by. Sudden disappearances earn the crab its name of Ghost.

pursuer passes it by. Then out it comes to race back toward more familiar beach.

It is difficult to decide whether a ghost crab becomes used to being handled. Cowles summarized his findings with the statement that the crab "has memory, is able to profit by experience, and can form habits." Certainly specimens selected at random on the beach and kept in

captivity adapt themselves to limited quarters and show no sudden awareness of freedom when the cage top is removed. This latter may be due to the indistinct vision on which Cowles experimented. Similarly, ocypodes which have been held by their chelae for a few minutes become quieter, and make only occasional attempts to escape if one chela is still held. For rather obvious reasons, the writers held the larger nipper in every instance. On one occasion, such a partially free crab reached over with the smaller pincer and seized the finger holding the large chela. The sudden nature of this counter-attack and the fact that the claw pierced the skin led the experimenter to let go of the large chela. The crab did not relax the smaller nipper but autotomized the whole appendage and raced away, brandishing its large pincer. The autotomized appendage clung to the finger for almost a minute before relaxing and falling to the sand. Meanwhile the crab had vanished down a hole in the beach and was free, presumably able to regenerate its lost appendage.

To observe ocypodes in captivity, glass culture dishes ten inches in diameter and four deep were separated into their two component halves, as Petri dishes can be. Each half was filled to a depth of two inches with very dry, clean beach sand and enough sea water was added to show through the thin glass side walls as darker, wetted sand. Single ghost crabs were kept in each such dish, with two or three layers of cheesecloth as a cover, held in place by strong elastic bands. On the first night each crab attempted to burrow into the sand over and over, so that by morning the earth was completely churned and uniformly damp. During the following day, each crab studied by the authors restricted its activity to one part of the dish, and hollowed out a short groove just inside the curving glass sidewall, the groove being as deep as the crab's body and provided with a good ramp at each end. By the second night, some crabs had become accustomed to digging the groove down to the bottom of the dish, and were able to excavate short, U-shaped burrows with a thin roof

and a ramp at each end. The burrow still followed the curve of the glass and was almost free of sand against the sidewall of the dish. On third and following nights, crabs which had been fed and allowed some exercise began their U-shaped burrows at the side of the glass even under observation and the hot glare of photoflood lamps. In only one specimen, and then after ten days in the dish, was a U-shaped tunnel constructed away from the sidewall, in the center of the sand area. These data seem to indicate a progressive adaptation by the crabs, to the new and very unusual living conditions in captivity.

When the culture dish covers were removed, the strong lights directed at the sand, and some activity jarred the table and dish, captive crabs would come slowly out of their burrows and explore the open dish area. Reaching a sidewall an inch or two high, ocypodes usually climbed over slowly and would drop from the rim if not prevented from doing so. Dropping to the table top (four inches) usually excited the crab so that it raced away, and again might receive a fall to the floor if the experimenters were unable to corral it and return it to the dish. One specimen which tumbled overboard landed on its back on the rung of a chair. Internal damages which were not evident externally led to its death within a few hours. Other specimens survived such accidents by falling on their legs or ventral surfaces, and were even more active after the additional stimulation. On the floor the crabs behaved much as on the beach, but showed a strong tendency to crawl vertically at any wall surface. Such behavior led to specimens climbing into radiators, and to clambering up heavy draperies to a height of as much as two feet. No corresponding activity was noted at the beach, although crab tracks were found each morning extending over dunes of medium height.

Cornered crabs always met approach of hands with outstretched chelae ready to seize and pinch. However, hands slid flat along the floor, palms up, could be manoevered in such a way that the crab would climb upon one

or the other. Under such circumstances no attempt was made to use the chelae, the sharp tips of the ambulatory appendages giving the crab such an excellent hold that the hand might be inverted with no danger of the crab falling off. Crabs picked up in this way would usually run slowly across the palm and could be kept in motion on first one hand, then the other. Similar technique was used to handle crabs which clambered out of the dish. Letting them run onto a palm, thence to a second palm, and from there back to the dish, gave both crab and experimenter some exercise. After a few dozen such sorties, crabs were usually content to rest awhile on the sand in the dish.

When relatively quiet, captive crabs would accept insects (dead or alive, dry or fresh) or other animal matter from a pair of forceps. Either or both chelae were used to seize such prey and carry it to the mouthparts. Large food such as whole Hippa (up to 3 cms long) was accepted eagerly by hungry crabs, and living pieces were ripped out by one nipper while the other held the meal securely. When non-fibrous anatomy such as gonads was reached, ocypodes usually employed the smaller chela with great rapidity, transferring tiny particles to the mouthparts faster than the eye could follow. Tough connective tissues were parted by stretching the prey between the two chelae and bringing the mouthparts to bear directly on the fibers under tension. Commonly a whole Hippa was too large a meal for a crab. The residue was dropped by some crabs, and carried into the burrow by others. No attempt to orient Hippa was seen, although such behavior was anticipated on the basis of Cowles's experience with small fish. Insects, on the other hand, were turned around very expertly in the chelae. First the head was devoured, then the body was reversed and the wings stripped back while the abdomen and thoracic tissues were devoured much as a person would eat a banana from the skin. Wings and other hard parts of such food were usually dropped very promptly. Any small insect was acceptable

prey, with the sole exception of fireflies (*Photuris* was the genus most frequently offered and declined). Even very hungry crabs would push a firefly away with the same violent outflinging of the chelae shown by a full-fed crab which is offered another morsel.

Cold sea water (at about 12° C.) offered to captive crabs was accepted from a medicine dropper brought up to the mouthparts. The water stimulated crabs to activity of the maxillipeds and frequent wiping of the eyestalks, but no resistance to added water was shown until excess was dripping from between the leg bases. Frequently crabs showed a curious gentle waving gesture with the smaller chela all during the administration of such cold water. However, the dropping of water on the back or eyes or legs called forth strong avoiding reactions and sometimes darkening of the chromatophores. The same results were obtained when a crab which had fallen on the floor or was too sand-covered for photography was rinsed in a saucer of cold sea water. As such crabs warmed up again under the radiant heat of the lamps, their chromatophore patterns became obliterated. Tests were made to show that it was a temperature change and not merely one of wetting the dorsal surface of the cephalothorax which brought about the change in color there. Specimens which were kept dry above with great care became darker when resting in a shallow dish of cold sea water. No harmful effects of this cold sea water were noted, although as mentioned above, cooling the whole crab to 12° C. brought on premature death in a matter of hours. Ocypodes which had been rinsed in cold sea water soon quieted down when returned to the dish, but specimens rinsed in fresh water of the same temperature became violently active and did not calm down for hours unless sea water was added to fill their gill chambers.

On one occasion, in order to handle a pair of ghost crabs with impunity, their chelae were tied shut with fine cotton thread. Looping the thread around the teeth on a claw kept it from slipping down the tapered nipper. The crabs

so tied were remarkably quiet. When handled, the chelae were pushed gently against the experimenter's fingers and much less attempt to escape was observed than in untied crabs. It should be noted, however, that most crabs with trussed chelae freed themselves within half an hour by bringing the threads to the mouthparts and chewing through the strands until they broke. Such animals re-

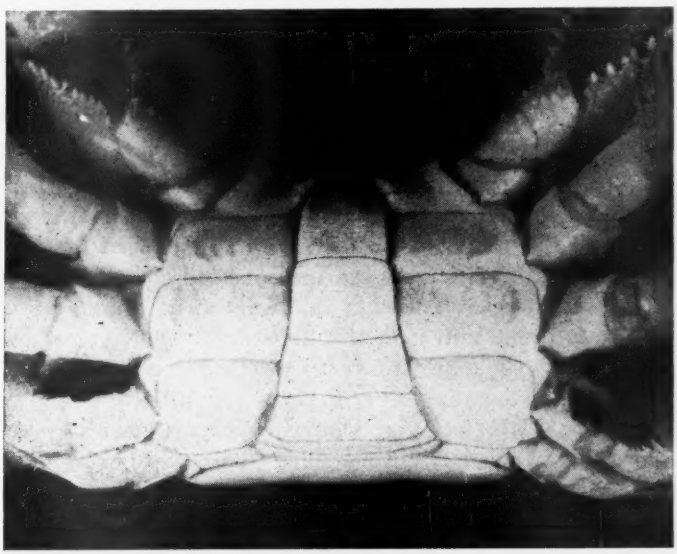

FIG. 5. Ghost crabs are modified for land life and can be drowned easily if confined in sea water. Between the 3rd and 4th pairs of legs is a hair-fringed opening into the breathing chamber. Appendages on the leg bases form fan blades to create a current of air in through these filters and out between the many mouthparts. A freshly wet crab froths at the mouth for some time.

covered their former reactions as soon as able to use their nippers.

Falling with subsequent bruising, wetting with fresh water and the initial handling which follows capture are three situations to which crabs react strongly. One additional should be mentioned—the shadow reaction. As noted above, the ghost crabs showed little or no concern

over the bright lights used in photographing their activities, but when these lights were turned off, all crabs jumped and ran. A shadow cast on them in the bright light had the same effect, and care must be taken not to produce such nervous responses if further observations in captivity are planned for the next many minutes.

Although *Ocypoda quadrata* has a well-developed stridulating ridge and row of denticles, no stridulation was observed by the present writers. It was looked for on the basis of the descriptions by Alcock (1892), Anderson (1894), Cowles (1908) and others, but no indication of such activity could be discerned. The popping of air bubbling through water among the mouthparts is an audible sound characteristic of the ocypodes examined. On a few occasions, a ghost crab startled on the beach emitted a short, sharp "peep" which may be produced by suddenly expelling air from the spacious gill chambers. No other sounds were noticed.

The present notes on the ghost crab's behavior, together with the many interesting details described in the literature listed, may serve to draw attention to *Ocypoda quadrata* (Fab.) as a highly developed crustacean suitable for study in what might be called "salt water terraria." It offers an excellent example of protective coloration and habits in a littoral animal, and adapts itself to captivity as well as, say, the horned toad, *Phrynosoma*. The ghost crab is common on sandy beaches from Atlantic City, N. J., southward, and furnishes an excellent and informative subject for observations in its natural environment.

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REVIEWS AND COMMENTS

EDITED BY PROFESSOR CARL L. HUBBS

In these reviews and notices of current biological publications emphasis is given to books and major articles which fall within the special scope of THE AMERICAN NATURALIST, in that they deal with the factors of organic evolution. REVIEWS AND COMMENTS are meant to include also such general discussions, reports, news items and announcements as may be of wide interest to students of evolution. Except as indicated, all items are prepared by Dr. Carl L. Hubbs, Scripps Institution of Oceanography, University of California, La Jolla, California. All opinions are those of the reviewer.

General Biology and Philosophy of Organism. By RALPH STAYNER LILLIE. Chicago: University of Chicago Press, 1945: 1–215. $3.00.

IN this book Lillie recapitulates, expands and synthesizes contributions that he has previously published in such journals as THE AMERICAN NATURALIST, *The Journal of Philosophy* and *Philosophy of Science.* As in those papers his integrating theme is that life processes are determined by "psychophysical" or perceptive as well as by purely physical factors. By an initial qualification of terms he might have used "psychical" in place of "psychophysical" for he admits that all life activities are physical in operation though they are not all attributable to the physical in ultimate analysis. In all organisms the physical and the psychical are regarded as inseparable and complementary. It is implied that the living system is an evolutionary derivative of the nonliving and it is admitted that some functions long supposed to be strictly vital are now known to be physical. Nevertheless the two "aspects" of life are strongly contrasted. Physical phenomena are characterized by stability, changeless with time, whereas psychical phenomena are held to involve novelty and creativity. For this reason the phenomena peculiar to life are always confined strictly to present time. Here I can't follow the author. His argument is that since present time is *new* and since the psychical as opposed to the physical is operative only in the present, "the psychical would appear to provide the required source of novelty."

By such logic and perhaps by the juggling of definitions and concepts the directive factor in both ontogeny and phylogeny is held to be psychical. Differentiation in this view acts in some respects not in accordance with but in opposition to physical laws. When in senility the directive, integrating psychical factors wane, the basic physical principle of entropy comes into play, whereupon death and disintegration ensue.

To render it obvious that the author's evolutionary philosophy has not been misinterpreted the following sentences are extracted from his book:

> Random variation . . . seems insufficient to account for the chief facts of evolution; and the entrance of teleological factors seems a more likely origin for many kinds of variation, especially the highly adaptive. . . . According to the foregoing view, evolutionary advances having special importance or value would be chiefly the outcome of psychical innovation. . . . The tendency toward novelty, syntheses and higher organization, as seen both in individual development and in evolution . . . is the expression of a directive and integrating factor which is psychical in its essential nature, although acting within or through the physical.

These are certainly not Neo-Darwinian precepts. Supported by increased estimates of world time most biologists do regard variation as essentially random rather than as adaptive. How psychical factors may have determined the nature of variation and of evolution is not clarified, nor is the "essential nature" of the psychical made clear. We may be considering an important treatise on philosophy, but not, I think, a particularly stimulating contribution to biology.

The Nature of the Beast. A Popular Account of Animal Psychology from the Point of View of a Naturalist. By RUTH CROSBY NOBLE. Based largely on the scientific publications, lectures, and notes of the late G. KINGSLEY NOBLE. Garden City, N. Y.: Doubleday, Doran and Co., 1945: i–xi, 1–224, 8 pls. $2.25.

ONE of America's foremost naturalists, G. Kingsley Noble, devoted most of his research energy to a study of those aspects of animal behavior that are related to life history, ecology and systematics. He had planned, we

are told, to coordinate his diverse, extensive and illuminating researches, along with the results obtained by others, into a general, readable treatise on the behavior of animals; but his untimely death intervened. This task, fortunately, was assumed by his wife, who had always been his intellectual companion. The result is not only a faithful tribute to her able husband but also a noteworthy and needed contribution to the popular literature of biology. The fact that such leading authorities as Robert M. Yerkes and Frank Beach read and criticized the manuscript goes far to assure its soundness.

After introducing the reader to the animal's point of view, Mrs. Noble discusses senses, instincts, learning and intelligence, all in a broadly comparative way. The evolution of animal societies, the establishment of social rank, and the holding of territories are then presented, with clarifying examples. Sex recognition, courtship and parenthood—topics particularly studied by Dr. Noble—are accorded emphasis in outstanding chapters. The discussion of parental care is weakened by the failure to consider fishes, which illustrate these phenomena particularly well. Sexual selection is rather discredited but then admitted as a probable factor in the evolution of sexual ornamentation. The "surplus energy" theory is advanced as the modern explanation of play but in the discussion that follows play is interpreted as the preparation for more serious adult activities.

Special creation, predetermined and purposive evolution, and the inheritance of acquired characters are summarily denied as factors in the evolution of behavior patterns. A strong Neo-Darwinian attitude is taken. "The same evolutionary processes which mould the form of a species are likewise responsible for evoking its characteristic behavior." Preadaptations, however, are held to be more effective than ordinary adaptations. The habitation of caves by the blind salamander of the Ozarks is oddly attributed to a temperature response. "Learned habits, though not in themselves inherited,

nevertheless may lead an animal into new environments and steer evolution along new channels." It is further concluded that some species are distinguished solely by behavioristic traits and that behavior as well as structure indicates relationships.

Students as well as the general public will profit from reading this book. Researchers will frequently consult it to obtain the welcome summary of Dr. Noble's views.

Meet Your Ancestors. A Biography of Primitive Man. By Roy Chapman Andrews. New York: The Viking Press, 1945: i–xiii, 1–259, 20 figs. and endpaper design. $3.50.

Decades of experience, as curator and director in a museum that has been notably responsive to modern interest-appeal, prepared Roy Chapman Andrews for this popular presentation of the story of human evolution. After the style of a museum diorama he reconstructs vividly the appearance and life ways of extinct men, in passages that are meant to be convincing though they are admittedly imaginative. He sugar-coats the text by playing up very interestingly the drama that was involved in the discovery of remains of each main kind of primitive man. Frequent anecdotes, based on his own connections with recent discoveries of fossil men, particularly in Asia, and on his close personal relations with leading physical anthropologists, surround the presentation with the air of a pleasant fireside conversation. Technicalities are avoided and controversies are subdued.

By such literary means the major facts and conceptions regarding the phylogeny of prehistoric men are made available to a larger audience than was reached by Raymond Murray in *Man's Unknown Ancestors* or by William Howell in *Mankind So Far*. The coverage is rather complete. Technical accuracy, a modern viewpoint and the inclusion of the latest findings (as the discovery of giant men in southeastern Asia) are assured by the author's reliance on the writings and personal advice of leading anthropologists, particularly Franz Weidenreich.

A few rather obvious slips of writing or of printing catch the eye.

The evolutionary point of view that is expressed in this book will surprise many biologists. There is an implied leaning toward Lamarckism in such sentences as "hanging by the arms straightened the body and produced internal adjustments" and "in living anthropoids the tail disappeared . . . probably because they habitually sat on their rear ends." An implicit belief in orthogenesis is indicated by the deletion of qualifications in the prediction that man, if he does not destroy himself, will inevitably become a tall, almost globeheaded, hairless creature with defective senses, 26 teeth requiring constant attention and only 4 toes on each foot.

Notices of New Books

The Ring-necked Pheasant and Its Management in North America. Edited by W. L. McAtee. Washington: American Wildlife Institute, 1945: i–xi, 1–320, frontisp., figs. 1–12, pls. 1–31. $3.50 in U. S.—Begun as a report by Daniel L. Leedy and Lawrence E. Hicks on the ecology, life equation and management of the ring-necked pheasant in Ohio, this treatise, augmented by accounts of pheasant research in other states, has grown into an impressive monograph on this introduced game bird. A particularly important chapter, by Howard M. Wight and students, deals with studies in Michigan, and the volume is dedicated to the memory of Professor Wight, pioneer teacher of wildlife managers. A historical introduction is contributed by the master conservationist Frederick C. Walcott, president of the American Wildlife Institute, which, along with the federal Fish and Wildlife Service, state conservation agencies and land-grant colleges, has supported a nation-wide program of wildlife research. A systematic synopsis of the genus *Phasianus*, with remarks on the extensive hybridization that has taken place between successively introduced kinds, is presented by Jean Delacour. Except in this chapter particular stress is paid to management practices that are designed to maintain an adequate stock of this favored game bird, but since wildlife researchers and administrators have come to realize that wise management of any

animal must stem from a thorough understanding of its ecology and life history, this book is also a treatise on the biology of the ring-necked pheasant.

Coral Fishes. By HYOZI AOYAGI [formerly Ikeda]. Part I. Tokyo: Maruzen Co., 18th year of the emperor = 1943: i–xii, 1–224, figs. 1–54, pls. 1–37 (7 col.).—Of the books on biology that were published in Japan during the war the first that has come to hand is a beautifully illustrated and carefully prepared descriptive treatment of the coral reef fishes of the Ryu-Kyu Islands. There are included some particularly detailed studies, as of dentition in eels and finer tooth structure in Hepatidae. The English is virtually faultless. As he tells us touchingly in preface and introduction, the author had planned to publish all his findings in a single volume, but was forced in great haste to prepare the first part for the press, before he was called to war. He dramatizes the soul-tearing conflict that arose within him, between an intense scientific spirit and a strong sense of social obligation, on the one hand, and a desire to serve and die for his country on the other hand. It is a happy circumstance that Aoyagi as well as Okada, Matsubara, Kuronuma and other Japanese ichthyologists survived the war, to cooperate with us in the reform and restoration of their lovely land and to continue to make known the extremely rich fish fauna of Japan.

Science and Scientists in the Netherlands Indies. Edited by PIETER HONIG and FRANS VERDOORN. New York: Board for the Netherlands Indies, Surinam and Curaçao, 1945: i–xxiv, 1–491, figs. 1–134. $4.00.—This large volume comprises an unorganized anthology of reprinted and original articles that touch on a wide variety of scientific fields. It is best characterized as an important contribution to geography. It indicates much of the scientific work that has been accomplished in the East Indies and covers or suggests much of the work that has been done on the geology, meteorology, agriculture, botany, zoology, physical anthropology and diseases of "Insulinde." There are interesting excerpts on explorations and chapters that treat the history and the significant accomplishments of scientific institutions in Java. Among the original contributions particular attention needs be directed to "The Geology of the Netherlands Indies," by H. STAUFFER; "Recent Studies on Sediments in the Java Sea and

Their Significance in Relation to Stratigraphic Petroleum Geology," by EARL H. MYERS; "Research on Fish and Fisheries in the Indo-Australian Archipelago," by ALBERT W. C. T. HERRE; "Wild Life Conservation in the Netherlands Empire, Its National and International Aspects," by J. H. WESTERMANN; and "The Puzzle of Pithecanthropus," by FRANZ WEIDENREICH. The last-cited article is an expert attempt to organize and interpret the fragmentary yet surpassingly significant evidence that has come from Java, bearing on the evolution of man. Although we recognize that Weidenreich is a cautious, critical and judicious, as well as highly competent worker, we can not regard as justified his unqualified assurance that the series of human fragments from the Trinil Formation exhibit a single definite evolutionary line leading from primitive giants to smaller men of more modern type.

There is included a list of "Scientific Institutions, Societies, and Research Workers in the Netherlands Indies." A reprint of this section, interleaved with blank paper, may be obtained without charge from the Library, Board for the Netherlands Indies, Surinam and Curaçao, 10 Rockefeller Plaza, 14th Floor, New York 20, N. Y., or from Editor, *Chronica Botanica*, P. O. Box 151, Waltham 54, Mass.

A Naturalist's Scrapbook. By THOMAS BARBOUR. Cambridge, Mass.: Harvard University Press, 1946: i–x, 1–218, 17 pls. $3.00.—Published shortly after his death, Tom Barbour's fourth book carries on the tradition that was set by the others. It is similarly forthright and bold, as interesting as a chat with the *muy simpático* naturalist who knew the whole world and its wealth of living creatures. The approach again is personal and very pleasantly so. This volume differs from and supplements the other three in that it deals primarily with the history, administration and functions of natural history museums. These subjects are considered from a general and philosophical viewpoint as well as with particular reference to the three museums which were favored by Barbour's guiding hand. Particular emphasis is naturally and happily given to problems and personalities that have been associated with the "Agassiz Museum," as Tom liked to call the Museum of Comparative Zoology. With a mixture of humor and other emotions the author recounts his long and finally successful struggles to renovate the museums

in Boston and Salem, bringing them into public service and instruction and removing them from undesirable competition with the research functions of America's oldest and grandest university museum.

About one third of this volume, however, relates previously unwritten experiences of the author in field explorations and travels, with biological reflections that came from this field work. "The Swing of the Pendulum" deals with what Barbour thought was the too extreme abandonment of the admittedly overworked land-bridge explanations of faunal similarities between islands. This chapter includes a valuable summary, contributed by E. D. Merrill, on the biogeography of Wallacea, the insular region that connects the island appendages of Asia and of Australia. In "Mostly about Islands" there is an interesting and significant discussion of the author's recent discoveries regarding the rapid speciation of rather small populations of snakes that have been completely isolated since Glacial time on Atlantic coastal islands.

Rocks and Rivers. By ELLIS W. SHULER. Lancaster, Pa.: The Jaques Cattell Press, 1945: [i–xx,] 1–300, figs. 1–105. $4.00.—Ordinarily the limits of biology are not transgressed on these pages, but Shuler's presentation of the stage on which organisms play their ceaseless drama is so excellently adapted to the needs of the general reader as to call for notice and commendation. It provokes interest and provides a wealth of information without entering into involved technical detail.

SHORTER ARTICLES AND DISCUSSION

AN ESTIMATE OF THE NUMBER OF CELL GENERATIONS PRECEDING SPERM FORMATION IN *DROSOPHILA MELANOGASTER*

SINCE it is possible that mutation may occur at any cell division in the ancestry of a given gamete, the question of the number of such pregametic divisions has some bearing on many studies involving mutation rates. The present report attempts to provide an estimate of the number of divisions ancestral to an average spermatozoon in *Drosophila melanogaster*.

In the developing *Drosophila* egg the polar cells (primordial germ cells) are differentiated at the 256-cell stage, *i.e.*, after eight cleavage divisions (Huettner, 1923). Of the primordial germ cells originally present, only about 9–13 are incorporated into each definitive testis (Huettner, 1940). By the time of hatching one or two mitoses have occurred, so that the newly hatched larva possesses about 36–38 spermatogonial cells in each testis (Sonnenblick, 1941).

The history of the individual cells between this stage and the development of primary spermatocytes is not clear. It is known that primary spermatocytes are formed in groups of sixteen, each such group having developed through four synchronous divisions of a single spermatogonial element, and each such group of course giving rise to 64 spermatozoa (Pontecorvo, 1941; personal observations). The spermatogonial elements involved in the four divisions preceding formation of the spermatocytes will be termed "definitive spermatogonia" in the subsequent discussion.

A vigorous male *D. melanogaster* should produce at least 10,000–14,000 offspring (Duncan, 1930). Since a female utilizes about 20–30 sperm per fertilized egg (Kaufmann and Demerec, 1942), the production of this number of offspring would indicate that some 200,000–420,000 sperm are produced, or 100,000–210,000 in each testis. Actually the number is probably higher, since sperm formation does not cease at the end of the fertile period; sterility seems to be due to functional failure of the ejaculatory mechanism (Duncan, *op. cit.*).

Assuming a strictly dichotomous[1] division of the approximately

[1] The term "dichotomous" is used throughout this paper to designate a type of cell lineage in which the two products of any given division have the same fate, *i.e.*, undergo the same number of subsequent divisions up to the time of sperm formation.

40 spermatogonial elements present in the newly hatched larva, 11–13 subsequent divisions would be required to produce this number of sperm. This includes the definitive spermatogonial divisions mentioned above and the meiotic divisions of the spermatocytes. On such a basis, the total number of divisions ancestral to any given sperm would be 22, as follows:

8	cleavage divisions
2	mitotic divisions before hatching
6	predefinitive spermatogonial divisions
4	definitive spermatogonial divisions
2	meiotic divisions
22	

Auerbach (1941) arrived at this same figure as a minimum for the number of pregametic divisions in the male *Drosophila*. Despite the mathematical possibility of producing all the sperm in this number of divisions, I do not believe acceptance of this figure as a minimum is warranted.

On the basis of the above mechanism it would be necessary for every one of the original spermatogonia to pass through all these generations; no spermatogonial element could enter into the definitive stages before it was six generations removed from the parent cell originally present at the time of hatching. Strictly synchronous division of all cells would be out of the question, since this would lead to the presence of only one stage of spermatogenesis at any given time, whereas sperm formation is actually continuous, all stages being found in pupae over 24 hours old, and in adults (Philip, 1942; Duncan, *op. cit.*). Any scheme of division even approaching synchrony would necessarily give rise to very large numbers of predefinitive spermatogonia. Such large numbers are never found; throughout most of pupal and all of adult life the number of spermatogonia is small and relatively constant.

It is doubtful whether any pattern of asynchronous, but eventually dichotomous, division could maintain a constant production of seventh generation (definitive) spermatogonia, while at the same time maintaining a very low number of cells of the preceding six (pre-definitive) generations. It would also be surprising if this marked asynchrony could continue through six generations, at the end of which there would be a sudden shift to the high degree of synchrony found in the definitive divisions.

Moreover, any strictly dichotomous scheme, whether synchronous or asynchronous, must eventually lead to exhaustion of spermatogonial elements, with consequent cessation of sperm formation. But, as indicated above, the number of spermatogonia appears to remain essentially constant throughout most of pupal and adult life, with no indication of any cessation of production of spermatozoa.

Production of sperm by dichotomy thus appears highly improbable. A more likely mechanism is that of an apical cell or a group of "cambial" cells. The presence of a single apical cell is doubtful on the basis of the division rate which would be required to produce the number of spermatozoa present at the time of eclosion (Auerbach, *op. cit.*). It also seems unlikely that the approximately eleven primordial germ cells entering into the embryonic formation of the testis would differ from each other in respect to their potentialities, although it is by no means inconceivable that one of these would take on the characteristics of an apical cell because it was, by chance, the one which became situated at the apex of the developing testis. Harris (1929) states that ". . . a single primordial germ cell . . . multiplies during the ensuing two or three weeks to form, on the average, about half of all the mature spermatozoa to be found in that testis." The figures on which this statement is based are not given. The same remarks apply to the assumption of two apical cells as to the assumption of one, and personal cytological observations give no indication of the existence of only one or two apical cells, but rather of a larger number of "cambial" cells.

On the other hand, it is conceivable that the primordial germ cells undergo one or two divisions, all the products of which assume the character of cambial cells. Since one or two mitoses have occurred at the time of hatching, it could be suggested that the spermatogonia then present comprise the cambial group. Even a greater number of precambial divisions could be postulated; there is no necessity of correlating developments within the testis with any particular stage in the development or life history of the individual. However, if the arrangement is strictly cambial, the number of cambial spermatogonia could not exceed the number of predefinitive spermatogonia in the testis at any stage of development. I have examined a number of slides of pupal testes (generously loaned to me by Dr. Curt Stern, of the University of Rochester) and, although the groupings of the

definitive spermatogonia are not as clear as might be wished, it is my opinion that the number of non-grouped, therefore predefinitive, spermatogonia is quite small, and would approximate eleven more closely than it would any multiple thereof. Conversely, the number of predefinitive spermatogonia appears higher than would be expected if only one or two apical cells were present.

It therefore seems most logical to assume that the approximately eleven primordial germ cells which enter the testis in early embryogeny behave as cambial cells, each giving rise, at every division, to a definitive spermatogonium, which in turn, after four mitotic and two meiotic divisions, produces 64 spermatozoa. Obviously the spermatozoa formed at different periods will be preceded by differing numbers of cambial divisions. We have postulated that some 100,000–210,000 sperm are produced in each testis during the fertile period; 150,000 can be taken as an average figure. Therefore the average sperm *which is utilizable for fertilization* would be preceded by the same number of cambial generations as the 75,000th sperm formed in a testis. Assuming equal division rates of all cambial cells, each cambial generation would produce $11 \times 64 = 704$ spermatozoa. The 75,000th sperm would be produced by the $\frac{75{,}000}{704}$ th, or the 107th, cambial generation. The total number of generations ancestral to this sperm would thus be.:

8	cleavage divisions
107	cambial divisions
4	definitive spermatogonial divisions
2	meiotic divisions
122	total ancestral divisions.

J. A. Tihen

Department of Zoology,
University of Rochester

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STRUCTURAL CHROMOSOME VARIABILITY IN URBAN AND RURAL POPULATIONS OF *DROSOPHILA FUNEBRIS*

THE causes that bring about karyotypic differentiation of populations constitute one of the fundamental problems of evolution of the species karyotype. The method of analysis of the giant chromosomes in the salivary gland cells of Drosophila permits making direct cytological observations on the variability of chromosomes in nature. Dubinin, Socolov and Tiniakov (1936, 1937) and Dobzhansky and Sturtevant (1938) have demonstrated with the aid of that method that Drosophila populations are saturated with chromosomal variants, and have outlined some ways in which the karyotypes of these populations may diverge. Till now, the problem has been studied chiefly in its static aspect, namely, in natural populations which are the products of a relatively slow evolution. The American authors work chiefly with wild species which live in forests (*Drosophila pseudoobscura*, *D. persimilis*) and avoid the species the populations of which are influenced by man. However, human activities create conditions which may give rise to a rapid evolution of many organisms. A remarkable example of this are the cyanide-resistant races of scale insects (see Dobzhansky, 1941). Human activities (in this case the fumigation with hydrocyanic gas) have led to the formation of new and stable races within a few decades.

Chromosome inversions are subject to natural selection because they bind together whole blocks of genes. This has been demonstrated by Dobzhansky (1943), who observed changes in the concentration of inversions in populations of *Drosophila pseudoobscura*, and showed that these changes are connected with the annual climatic cycle. Our data on *Drosophila funebris* (Dubinin, Socolov and Tiniakov, 1937; also unpublished data) have also shown a dependence of the concentration of inversions on the seasonal breeding conditions of populations.

Drosophila funebris exists under different living conditions in urban and in rural localities. Human activities create distinct ecological complexes which form an integral part of the environment in which the evolution of the species is being enacted. The relatively recent appearance of modern industrial centers has evoked powerful evolutionary processes the study of which should be used as a method of studying evolution in general. Our observations have shown that populations of *D. funebris* that inhabit urban localities are clearly distinct in their karyotypes from those living in rural localities. Dubinin, Socolov and Tiniakov (1937) and particularly Tiniakov (unpublished) have found differences in the concentration of inversions in populations of different localities. However, the connection between these differences and the living conditions in the rural and urban

TABLE 1

DISTRIBUTION OF INVERSIONS IN DIFFERENT POPULATIONS IN THE CITY OF MOSCOW

Part of the city	Individuals studied	Number of inversion heterozygotes	Percentage of heterozygotes
Botanical Garden of the Moscow State University	501	303	58.48
Samotechnaia Square	900	797	88.54
Obukh Street (Voronzovo Polye)	869	439	50.52
Neighborhood of the Hippodrome	1,045	175	16.74
Total	3,315	...	53.83

communities has become apparent only as a result of the population analyses made in 1944 and 1945.

Eight different gene arrangements occur in the Moscow populations of *Drosophila funebris,* four of them in the second chromosome (the Standard and three different inversions), one inversion in the third, and one in the fourth chromosomes. The limits of the inversions are determined with the aid of Tiniakov's maps (unpublished). *In* II–1 extends from 23C to 31B; *In* II–2 from 29A to 32B; *In* II–3 from 32B to 34C; *In* III–1 from 50A to 56A; *In* IV–1 from 67C to 73A/B. The karyotypes of 3,315 individuals from the Moscow populations were determined during 1944–1945. Very high concentrations of inversions were found, which, in addition, were different in different parts of the city, as shown in Table 1.

The concentration of inversion heterozygotes in different Moscow populations varies from 17 to nearly 90 per cent., with an average of approximately 54 per cent. The examination of 2,196 individuals from rural localities around Moscow revealed

the same inversions which were found in the city (II–1, II–2, IV–1), but the concentration of the inversions proved to be quite different, as shown in Table 2.

The populations of Moscow are, thus, remarkable because of the enormous concentrations of inversions. This can hardly be due to accident, since species connected with man, such as *Drosophila funebris*, migrate very easily, and there can be no doubt that interchange of populations between Moscow and its environs is constantly going on. It is obviously important to find out whether the observed type of difference between urban and rural populations is observed only in and around Moscow or is a more widespread phenomenon. For this purpose, we collected and examined 231 individuals in the city of Ivanovo, and 288 individuals in nearby rural communities, in Liubim (150 km east of

TABLE 2

DISTRIBUTION OF INVERSIONS IN DIFFERENT RURAL POPULATIONS AROUND MOSCOW

Locality	Individuals studied	Number of inversion heterozygotes	Percentage of heterozygotes
Kropotovo (115 km southeast of Moscow)	1,375	19	1.39
Polivanovo (210 km southeast)	635	9	1.42
Khotkovo (60 km northeast)	186	3	1.61

Ivanovo) and Spaso-Shelutinskoe (90 km northeast of Ivanovo). In the city, 86 inversion heterozygotes were found (37.23 per cent.), but none were met with in the rural localities.

It is evident that urban environments offer favorable conditions for the spread of inversions, while in rural places the standard gene arrangements predominate. It is possible that the different conditions during hibernation play an important role here. At any rate, neither in urban nor in rural localities is there a complete uniformity of chromosome structure. It appears that in every population natural selection works in different directions, thus preventing the establishment of homozygosis. The urban and rural living conditions are different, and the differences between their populations are caused by natural selection. We have here for the first time a demonstration of the effects of natural selection by different living conditions on the process of karyotypical divergence of whole populations.

N. P. DUBININ
G. G. TINIAKOV

INSTITUTE OF CYTOLOGY OF THE ACADEMY OF SCIENCES OF USSR, MOSCOW

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EGG PRODUCTION IN LYMNAEA COLUMELLA[1]

Introduction

The hermaphroditic pulmonate gastropod, *Lymnaea columella*, can reproduce either by cross-fertilization or by self-fertilization when isolated (Colton, 1927; Crabb, 1927). There is an ovotestis, a gonoduct and an albumen gland which forms the jelly in which the eggs are laid, each surrounded by its own hyaline capsule or envelope. The jelly mass containing the eggs is contained within a tough, cylindrical sac, sealed at both ends and herein referred to as a "clutch." There were two problems to be solved:

(1) Is the production of eggs independent to some extent of the formation of the clutches, or are these activities the manifestation of a single biological control?

(2) Can the number of clutches be considered a reliable index of total productivity, since an extremely high correlation between the number of eggs and the number of clutches was found, or are there other characteristics of the life cycle which also have a significant relation to egg production?

Methods and Materials

A family of thirty-seven snails, obtained from a single clutch of self-fertilized eggs, was isolated at hatching in jars containing 500 ml of spring water. In so far as was possible, they were grown under uniform conditions. The temperature of the laboratory was kept approximately between 19° C and 25° C. The food consisted of fresh lettuce. Complete records of the laying cycle were obtained, and also of the age at death. These are given in Table 1.

[1] The author wishes to acknowledge her indebtedness to Mr. Daniel Rosen for his help in the statistical analysis of the data.

TABLE 1

	Age at onset	Days laying	No. clutches	Total no. eggs	Aver. no. eggs per clutch	Age at death
1	82	136	105	3578	34.08	220
2	90	58	44	2017	45.84	150
3	84	123	82	3055	37.25	214
4	83	133	98	3200	32.65	219
5	82	109	68	2713	39.89	194
6	84	131	97	3365	34.69	238
7	85	126	98	3326	33.94	213
8	84	121	76	2791	36.72	210
9	81	85	60	2460	41.06	173
10	85	89	67	3126	42.57	176
11	84	127	82	3146	38.36	214
12	83	125	77	3430	44.54	213
13	81	131	111	3538	31.87	215
14	81	97	67	2800	41.79	131
15	(81)	(27-inc.)	(15)	(536)	(35.73)	...
16	81	132	102	4084	40.04	219
17	81	94	67	2724	40.26	177
18	81	132	79	3148	39.86	207
19	81	96	65	2733	42.04	179
20	81	145	97	3575	36.85	235
21	84	125	91	2697	29.64	212
22	81	98	65	2109	32.44	180
23	80	90	55	2254	40.98	172
24	81	133	84	2810	33.45	227
25	86	81	51	1970	38.63	171
26	81	130	78	2580	33.08	216
27	87	116	82	3240	39.51	206
28	81	115	79	2014	25.49	200
29	82	122	80	2091	38.63	205
30	Sterile					
31	81	139	86	2913	33.87	227
32	81	121	82	2664	32.49	205
33	81	96	67	2340	34.92	180
34	81	136	94	2732	29.06	220
35	81	16	7	144	20.57	113
36	85	113	70	2839	40.55	203
37	82	114	96	3395	35.36	228
			2,709	98,602		

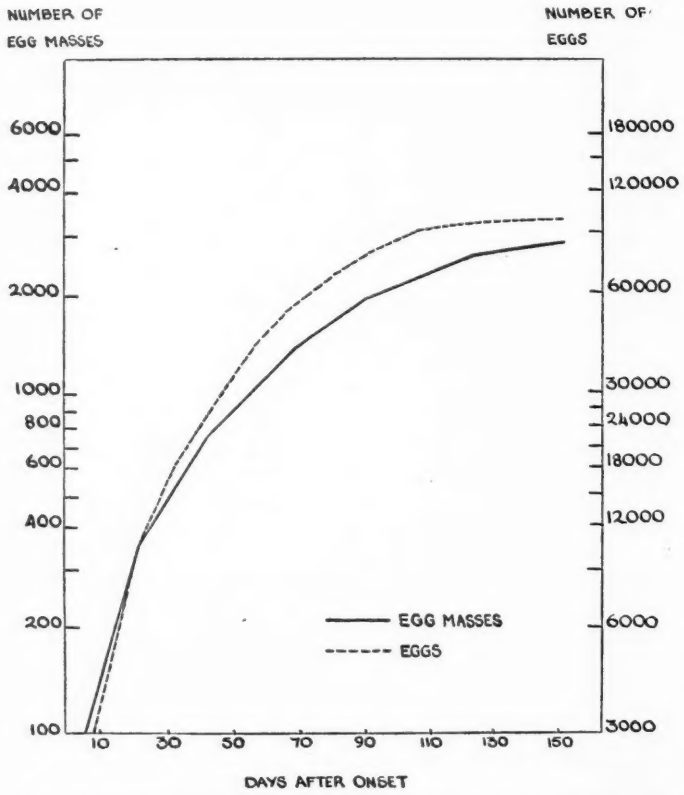
TABLE 2

Period in days	Cum. no. clutches	No. of clutches	Cum. no. of eggs	No. of eggs
10	138	138	3539	3539
20	306	168	9420	5881
30	474	168	15902	6482
40	691	217	24649	8747
50	913	222	33998	9349
60	1169	256	45753	11755
70	1429	260	57178	11425
80	1652	223	67086	9908
90	1950	298	78181	11095
100	2185	235	86451	8270
110	2355	170	91451	5000
120	2540	185	95936	4485
130	2683	143	98829	2893
140	2723	40	99368	539
150	2725	2	99389	21

Analysis: Problem 1

The clutches may, on rare occasions, contain nothing but jelly, but as a rule they contain a mumber of eggs which varies from one to over one hundred. Sometimes the envelopes hold, not the

usual solitary egg, but groups of eggs, perhaps as many as twenty-five. On the other hand, empty envelopes are sometimes formed, so that the clutch contains a combination of normal eggs and "empties." Colton (1908) describes these empty envelopes



Rate of growth of cumulative clutch production and cumulative egg production.

as occurring in decadent material, but in this family of snails, they have been observed to occur in vigorous snails, sometimes habitually. These wide variations suggest that the production of eggs and the formation of clutches are somewhat independent functions in reproduction.

To test this hypothesis, the correlation between the eggs produced and the clutches produced by the group was investigated; it was found to be + .87, a high "r" for biological material. These calculations were based on the daily laying records. When 10-day intervals were used as the unit periods, the correlation between the egg and clutch production was found to be + .93. This higher "r" may be due in part to the elimination of errors in estimating the age of the early embryos.

Cumulative egg production and cumulative clutch production were then plotted on semi-logarithmic paper so that the growth of the two functions could be observed comparatively. As shown in the chart, these curves are distinctive, yet they do not differ in form or in rate of change.

Analysis: Problem 2

The above calculations show beyond any doubt that egg production is related to clutch production, yet the two functions are sufficiently independent to indicate that they do not represent a univariate universe. Are there then perhaps other significant figures, which can be discovered to be operating in egg production? The available data are age at onset, average number of eggs per clutch, length of the laying period and age at death; variables arbitrarily chosen because the data are obviously important and can be accurately measured.

By multiple correlation technique an equation was worked out to express total egg production (after eliminating the factor of average number of eggs per clutch, because of the wide dispersion around the mean and age of onset and because this factor showed so little variation):

$$X'_{1.234} = 623.34 + 23.6X_2 + 6.94X_3 - 1.84X_4,$$

in which X' is the total number of eggs laid; X_2 is the total number of clutches; X_3 is the total number of days in the laying period; and X_4 is the total number of days in the life cycle.

The partial coefficients of estimation in the equation were converted into Beta coefficients, and the standard errors of the partial coefficients of estimation were calculated. The following conclusions were drawn:

(1) The number of clutches has a positive and exceedingly important relation to total egg production

(2) The length of the laying period is to a small but significant degree related to total egg production

(3) Length of life has no significant relation to egg production.

The negative sign of X_4 was investigated, but it was found to have no significance.

Since the addition of X_4 to the above equation does not, therefore, contribute to the explained variation, it may be eliminated from the estimating equation, which was then computed to be:

$$X'_{1.23} = 429.85 + 21.74X_2 + 6.66X_3.$$

The standard error of estimate was computed to be 323.

It is clear that egg production is correlated not only with clutch production, but also with the length of the laying period. An estimate of productivity in a large sample of snails can therefore be made, using these two variables, thus eliminating the enormous labor of counting each egg in studies of variation.

It should be noted that Bailey (1939) also found that longevity is not related to fecundity in the species. The family of snails described here, however, had a higher egg production than did those studied by this author. Succeeding generations of the Barnard snails exhibited this same difference consistently, suggesting the possibility that two different strains, with a different genetic constitution, may have been used in the two investigations.

Summary

Egg production and clutch production in *Lymnaea columella* were found to be directly related. The correlation, on the basis of the daily records, was +.87; on the basis of 10-day periods, it was +.93. A second factor, the length of the laying period, was also found to be to a small, but significant extent related to egg production. Longevity was found not to be related to productivity.

Grace Springer Forbes

Barnard College,
Columbia University

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